Verigene® Gram-Negative Blood Culture Nucleic Acid Test (BC-GN)

IVD

Rx Only

20-005-021 (Test Kit) ● 20-012-021 (Utility Kit)

KEY-CODE **NAN021**

INTENDED USE

The **Verigene® Gram-Negative Blood Culture Nucleic Acid Test (BC-GN)** performed using the sample-to-result Verigene® System, is a qualitative multiplexed *in vitro* diagnostic test for the simultaneous detection and identification of selected gram-negative bacteria and resistance markers. **BC-GN** is performed directly on blood culture media using blood culture bottles identified as positive by a continuous monitoring blood culture system and which contain gram-negative bacteria as determined by Gram stain.

BC-GN detects and identifies the following:

Bacterial Genera and Species	***Resistance Markers***
Acinetobacter spp. *Citrobacter* spp. *Enterobacter* spp. *Proteus* spp. *Escherichia coli* [1] *Klebsiella pneumoniae* *Klebsiella oxytoca* *Pseudomonas aeruginosa*	CTX-M (*bla*$_{CTX\text{-}M}$) KPC (*bla*$_{KPC}$) NDM (*bla*$_{NDM}$) VIM (*bla*$_{VIM}$) IMP (*bla*$_{IMP}$) OXA (*bla*$_{OXA}$)

[1] **BC-GN** will not distinguish *Escherichia coli* from *Shigella* spp. (*S. dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei)*

BC-GN is indicated for use in conjunction with other clinical and laboratory findings to aid in the diagnosis of bacterial bloodstream infections; however, is not used to monitor these infections. Sub-culturing of positive blood cultures is necessary to recover organisms for antimicrobial susceptibility testing (AST), for identification of organisms not detected by **BC-GN**, to detect mixed infections that may not be detected by **BC-GN**, for association of antimicrobial resistance marker genes to a specific organism, or for epidemiological typing.

BACKGROUND INFORMATION

Bloodstream infection (BSI) occurs when a pathogenic microorganism, such as a gram-negative bacterium, enters the bloodstream. **BC-GN** is a multiplex, automated nucleic acid test for the identification of the genus, species, and genetic antimicrobial resistance determinants for a selected panel of the most common gram-negative blood culture bacteria. While detection of BSI with conventional microbiological methods may require 2-4 days to produce bacterial identification and resistance results, **BC-GN** provides results within 2 hours of blood culture positivity.

The Verigene System's unique instrumentation allows for random access test processing, enabling on-demand testing directly from positive blood culture bottles without the need for batched testing. A brief description of the organisms detected by **BC-GN** ("**BC-GN** panel members") and their clinical relevance follows.

Acinetobacter spp. is a genus of bacteria that are strictly aerobic nonfermentative gram-negative bacilli. Species of *Acinetobacter* exhibit a broad degree of antibiotic resistance, including inherent resistance to many classes of antibiotics such as penicillin, chloramphenicol, and often aminoglycosides.[1] Increasingly recognized as a major pathogen causing nosocomial infection, including bacteremia, *Acinetobacter baumannii* is emerging as a cause of numerous global outbreaks due to multi-drug-resistant (MDR) strains, making it very difficult to treat. In *A. baumannii,* carbapenem resistance may result from carbapenemases such as OXA class D beta-lactamases, metallo-beta-lactamases (MBLs), and the Ambler class A *K. pneumonia*e carbapenemases (KPCs) .[2,3,4]

Citrobacter spp. is a genus of gram-negative bacteria belonging to the family *Enterobacteriaceae* that are found in soil, water, and wastewater. This bacterium is an opportunistic pathogen that is rarely the source of illness, except for infections of the urinary tract, infant meningitis, and sepsis.[5] A high degree of antibiotic resistance is noteworthy for *Citrobacter freundii* and *Citrobacter koseri*. Numerous international molecular studies have described the occurrence of various CTX-M types in *Citrobacter* spp.. KPC-harboring isolates have been described in the United States, Asia, and Europe.[6,7,8,9]

Enterobacter spp. is a genus of gram-negative bacteria also belonging to the family *Enterobacteriaceae* that are an opportunistic cause of nosocomial bloodstream infections. Several strains of *Enterobacter* are pathogenic and cause infections in immunocompromised patients and those that require mechanical ventilators. The two clinically relevant species of *Enterobacter* are *Enterobacter aerogenes* and *Enterobacter cloacae.* Various resistance mechanisms have been described in *Enterobacter*, including production of carbapenemases and other extended-spectrum beta-lactamases (ESBLs).[10,11]

Proteus spp. is a genus of gram-negative bacteria belonging to the family *Enterobacteriaceae* that are an opportunistic human pathogen commonly found in decomposing animal matter, sewage, and human and animal feces. Three species are opportunistic human pathogens: *Proteus mirabilis*, *Proteus vulgaris*, and *Proteus penneri*. *Proteus mirabilis* is the most common *Proteus* species isolated in blood. Like many members of the family *Enterobacteriaceae*, *Proteus* spp. can harbor numerous antimicrobial resistance determinants, including ESBL. Carbapenem resistance mediated by carbapenemases such as OXA, MBL and KPC has been reported. [12]

Escherichia coli is a gram-negative bacterium belonging to the family *Enterobacteriaceae* that is the most common cause of gram-negative bloodstream infections.[13] Most strains of *E. coli* are harmless and part of the normal flora of the gut. Virulent strains of *E. coli* can cause gastroenteritis, urinary tract infections, and in rarer cases, hemolytic-uremic syndrome and septicemia.[14] This bacterium is associated with significant mortality if it reaches the bloodstream and is responsible for approximately 8% of all bloodstream infections.[13] *E. coli* may produce ESBL, KPC, OXA-48, or NDM-1.

Klebsiella pneumoniae is a gram-negative bacterium belonging to the family *Enterobacteriaceae* and is clinically the most significant member of the *Klebsiella* genus. *Klebsiella pneumoniae* is closely related to *Klebsiella oxytoca*, through which it is distinguished by being indole-negative and by its ability to grow on both melezitose and 3-hydroxybutyrate. The most common infection caused by *Klebsiella pneumoniae* is pneumonia, typically in the form of bronchopneumonia and bronchitis, but can also escalate to septicemia. Feces are the most significant source of patient infection, followed by contact with contaminated instruments. This bacterium may produce KPC, OXA, or NDM-1. Carbapenemase-producing *Klebsiella pneumoniae* is an emerging challenge in health-care settings, with a progressive increase seen worldwide over the past 10 years. Carbapenem-resistant *Klebsiella pneumoniae* are resistant to almost all available antimicrobial agents and these infections have caused high rates of morbidity and mortality.[15]

Klebsiella oxytoca is a gram-negative bacteria belonging to the family *Enterobacteriaceae* that is found in a wide range of environments and are opportunistic in nature. Most infections with *Klebsiella oxytoca* are nosocomial, with outbreaks occurring in immunocompromised patients being treated with antibiotics. *Klebsiella oxytoca* has increasingly been present in the blood samples of infants with neonatal septicemia.[16] These bacterium can produce KPCs.

Pseudomonas aeruginosa is a gram-negative bacterium that is an opportunistic human pathogen commonly found in soil, water, skin flora, and most man-made environments. *Pseudomonas aeruginosa* is the third most common causative pathogen of gram-negative bloodstream infections and has higher virulence than *Klebsiella* species. Patients with septic shock caused by *Pseudomonas aeruginosa* display a purple-black skin lesion *ecthyma gangrenosum*. *P. aeruginosa* shows a high level of intrinsic resistance to antimicrobial drugs and an ability to become even more drug resistant. These bacteria can acquire resistance with KPC and ESBL production. In addition, outbreaks by MBL-producing *P. aeruginosa* have been documented in hospitals in several countries, with VIM being the most dominant MBL variant worldwide.[17]

The treatment of serious bacterial infections in clinical practice is often complicated by antibiotic resistance. Resistance rates are increasing among several problematic gram-negative pathogens that are often responsible for serious nosocomial infections, including *Acinetobacter* spp., *Pseudomonas aeruginosa*, and *Enterobacteriaceae*. The presence of multi-resistant strains of these organisms has been associated with prolonged hospital stays, higher health care costs, and increased mortality.[19] A brief description of the genetic resistance determinants detected by **BC-GN** follows.

CTX-M: CTX-M-type enzymes are a group of class A ESBLs that are rapidly spreading among *Enterobacteriaceae* worldwide, replacing TEM-type and SHV-type ESBLs as the predominant ESBLs in many countries. Some carbapenemases have become associated with strains that have great epidemic potential, spreading across countries and continents. [22,23,24] This enzyme is acquired through the plasmid acquisition of beta-lactamase genes rather than via genetic mutation. There are more than 80 CTX-M enzymes that have been identified. [20,21]

KPC: The class A KPC has rapidly spread in the United States and is increasing elsewhere in the world. At least ten variants have been identified and are distinguished by 1-2 amino acid substitutions.[8] Class B metallo-beta-lactamases (MBLs) of the IMP, VIM, and NDM types have been reported worldwide, and their genes are carried by plasmids and integrons, hydrolyzing all beta-lactams with the exception of aztreonam.[25]

NDM: New Delhi metallo-beta-lactamase (NDM) is a novel broad spectrum carbapenemase. Since its first description, NDM carbapenemases--with NDM-1 presenting as the dominant type--has been reported from many countries worldwide. NDM enzymes are present largely in *Enterobacteriaceae*, but also in non-fermenters.[26,27,28]

VIM: Verona integron-encoded metallo-beta-lactamase (VIM) belongs to a growing family of carbapenemase enzymes that includes at least 10 known members, of which VIM-2 is the predominant variety. VIM enzymes have been found mainly in non-fermenting gram-negative bacteria such as *Pseudomonas aeruginosa*, but their numbers are increasing in *Enterobacteriaceae.*[25]

IMP: Imipenem-resistant metallo-beta-lactamase (IMP) is a plasmid mediated IMP-type carbapenemase, with at least 17 known varieties. IMP enzymes are most frequently seen in *Pseudomonas* and *Acinetobacter* species.

OXA: Class D beta-lactamases are also called oxacillinases, or OXA-type beta-lactamases (OXAs). Genes encoding OXAs are known to be intrinsic in many gram-negative rods, including *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, but play a minor role in natural resistance phenotypes. The acquired OXAs possess either a narrow spectrum or an expanded spectrum of hydrolysis, including carbapenems in several instances. None of these carbapenem-hydrolyzing class D beta-lactamases (CHDLs) significantly hydrolyze expanded-spectrum cephalosporins, therefore indicating that currently known OXAs are unable to combine extended-spectrum and carbapenem-hydrolyzing properties. Three groups of acquired CHDLs (OXA-23-like, OXA-40-like, and OXA-58-like) have been identified in *Acinetobacter* species. OXA-48-like enzymes, another CHDL, are mostly identified in *K. pneumoniae* and *E. coli*, but also in various other enterobacterial species.[29,30,31]

PRINCIPLES AND PROCEDURES OF BC-GN AND THE VERIGENE SYSTEM

BC-GN is performed using the Verigene System, which is a bench-top sample-to-result molecular diagnostics workstation consisting of two modules: the Verigene® Processor *SP* and the Verigene® Reader. The Verigene Processor *SP* automates the **BC-GN** sample analysis steps including: (i) Specimen Preparation - cell lysis and magnetic bead-based nucleic acids extraction from positive blood culture specimens and (ii) Hybridization—bacterial DNA hybridization to target specific capture DNA in a microarray format and mediator and gold-nanoparticle probe hybridization to captured bacterial nucleic acids. Silver enhancement of the bound gold nanoparticle probes at the capture sites results in gold-silver aggregates that are imaged optically with high efficiency by the Verigene Reader. The Verigene Reader also serves as the user interface and central control unit for the Verigene System, storing and tracking information throughout the assay process.

The Verigene Processor *SP* utilizes single-use consumables to perform **BC-GN**, including an Extraction Tray, Utility Tray, and Test Cartridge. A separate Tip Holder Assembly contains two pipette tips that are used to transfer and mix reagents during the assay. The user tests a specimen by loading the single-use disposables into the Verigene Processor *SP*, pipetting the specimen into the Extraction Tray, and initiating the protocol on the Verigene Reader by scanning or entering the Test Cartridge ID and specimen information. Following assay completion, the user inserts the substrate slide portion of the Test Cartridge into the Verigene Reader for optical analysis and generation of **BC-GN** test results.

MATERIALS PROVIDED

. Verigene® **BC-GN** Test Kit (Catalog number **20-005-021**)

- 20 Verigene® **BC-GN** Test Cartridges
  Each Test Cartridge comes preloaded with all required reaction solutions, including wash solutions, oligonucleotide probe solution and signal amplification solutions required to generate a test result. The Test Cartridges are labeled as: **BC-GN; 20-006-021**

- 20 Verigene® **BC-GN** Extraction Trays (with Tip Holder Assemblies)
  Each Extraction Tray comes preloaded with all required solutions, including lysis/binding buffer, digestion enzymes, wash solutions, and buffer solutions necessary to extract nucleic acids and generate a test result. The Extraction Trays (with Tip Holder Assemblies) are contained within a carrier labeled as: **BC-GN; 20-009-021**

- 20 Verigene® Sample Well Caps
  The Sample Well Caps come packaged in strips of 5 Caps. The Sample Well Caps are contained within a plastic bag labeled as: **40-001-001**

. Verigene® **BC-GN** Test Utility Kit (Catalog number **20-012-021**)

- 20 Verigene® **BC-GN** Utility Trays
  Each Utility Tray comes preloaded with an Internal Processing Control. The Utility Trays are contained within a carrier labeled as: **BC-GN; 20-011-021**

MATERIALS NEEDED BUT NOT PROVIDED

. Instruments and Equipment

- Verigene Reader; Catalog number 10-0000-02
- Verigene Processor *SP*; Catalog number 10-0000-07
- 2–8 °C Refrigerator
- ≤-20 °C Freezer
- Automated blood culture monitoring system
- Micro-pipettors & tips
- Vortex mixer
- Biological Safety Cabinet (BSC) (Optional)
- Cartridge cover opener (Optional)
- Verigene® Extraction Tray Holder; Catalog number 421-00019-01 (Optional)
- ≤-70 °C freezer (Optional)

Consumables and Reagents

- Blood culture bottles
- Gram staining reagents

REAGENT STORAGE, HANDLING, STABILITY

Component	*Storage Conditions*	*Comments*
Extraction Tray	2–8 °C	Do not freeze.
Test Cartridge		
Tip Holder Assembly	2–30 °C	
Sample Well Caps		
Utility Tray	≤-20 °C	Shipped frozen. Upon receipt, store frozen. Avoid multiple freeze/thaw cycles.

VERIGENE DAILY MAINTENANCE

A. Work Area Preparation

Each day of testing and before and after sample preparation, prepare the testing work area by sanitizing the BSC (if utilized), countertops, vortex mixers, micro-pipettors, and any other equipment used for sample processing with a lint-free decontaminating wipe.

B. Verigene System Cleaning

Prior to the start of testing each day, perform the following steps for each instrument used for testing.

While wearing fresh gloves, use a lint-free decontaminating wipe to thoroughly wipe the drawer assembly of the Verigene Processor *SP*. For the Verigene Reader, wipe down the user Touchscreen, Barcode Scanner and the door of the Analysis Compartment. It is not necessary to change gloves between instruments; however, do not use the same lint-free decontaminating wipe to clean different instruments.

Please refer to the Verigene System User's Manual for additional details on performing tests on the Verigene System as well as routine and daily maintenance.

METHODS

A. Specimen Collection, Processing and Storage

Inadequate or inappropriate specimen collection, storage, or transport may yield false-negative results. Due to the importance of specimen quality, training of personnel in the correct manner to perform specimen collection and handling is highly recommended.

1. Draw blood using aseptic techniques into the blood culture bottle following manufacturer's instructions.
2. Incubate the bottle in an automated blood culture monitoring system until the bottle is flagged positive for microbial growth following manufacturer's instructions.
3. When the bottle is positive for microbial growth, perform a Gram stain.
4. For gram-negative bacteria, test 700 µL of the blood culture media using **BC-GN**. Ensure the blood culture bottle is thoroughly mixed by inverting several times (≥4) before retrieving test sample volume.
5. Sub-culturing of positive blood cultures is necessary to recover organisms for AST, for identification of organisms not detected by **BC-GN,** to detect mixed infections that may not be detected by **BC-GN**, for association of antimicrobial resistance marker genes to a specific organism, or for epidemiological typing.
6. Positive blood culture bottle media can be stored at 2-37 ºC for up to 24 hours after bottle positivity prior to testing.

B. Test Procedure

1. Test set up—after Specimen Processing

   a) Remove an Extraction Tray, Tip Holder Assembly and Test Cartridge from the refrigerator. Remove a Utility Tray from the freezer and begin test run within 30 minutes.

      Note: for Utility Trays stored at temperatures <-20 ºC, thaw the tray at room temperature for at least 10 minutes prior to beginning test run**.**

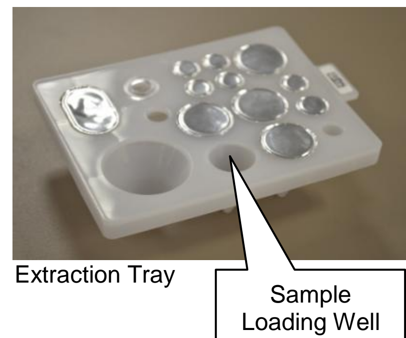
   Optional Cap Protocol:

   i. Remove one cap from the strip of Sample Well Caps and place inside the BSC.

   ii. Place the Extraction Tray in the Extraction Tray Holder inside the BSC. (Refer to image below for Extraction Tray Holder)

iii. *Pipette 700 µL of the prepared sample into the bottom of the Sample Loading Well in the Extraction Tray. (Refer to image below for Sample Loading Well location).*

Extraction Tray Holder

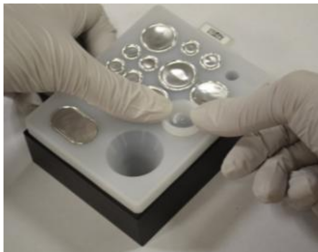


Extraction Tray

Sample Loading Well

iv. *After sample loading, place the Sample Well Cap over the Sample Loading Well. Take precaution to handle only the edges of the Cap and firmly press down until the Cap is fully inserted into the Sample Loading Well.*

Sample Well Cap in Packaging



Pressing down on edges of cap

Extraction Tray with cap inserted

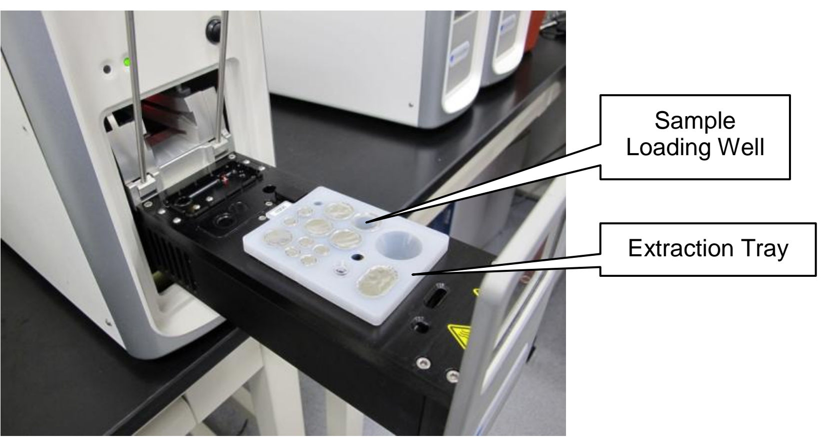
v. *Take the Extraction Tray out of the BSC and insert into the Extraction Tray Module on the SP.*

b) The image below shows an empty Verigene Processor *SP*. Open the Drawer Assembly by pressing the black OPEN/CLOSE button located on the front of the Verigene Processor *SP*. Open the Drawer Clamp by pressing in the silver latch and lifting the Drawer Clamp prior to loading the consumables.

2. Loading the Extraction Tray

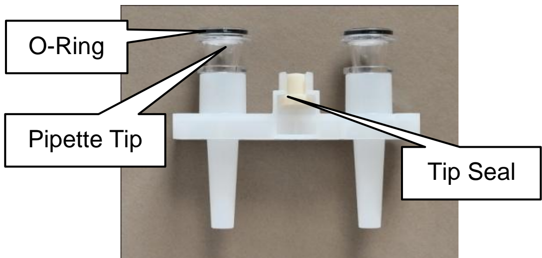
   a) (*optional*) Prior to loading the Extraction Tray, thoroughly shake the Extraction Tray to resuspend the magnetic beads that have settled during storage. Check for complete resuspension by visually inspecting the well containing the beads. The well containing the magnetic beads is easily distinguished, as the beads are black in color. Following adequate resuspension, gently tap the tray on the bench to ensure that the reagents settle to the bottom of each well.

   b) The Extraction Tray can only be loaded in one location and orientation in the Drawer Assembly. When the Extraction Tray is loaded correctly, the Sample Loading Well is located at the right hand side of the Drawer Assembly. Place the Extraction Tray in the Drawer Assembly and press down on the corners of the tray to ensure it is level. The image below shows a properly loaded Extraction Tray.



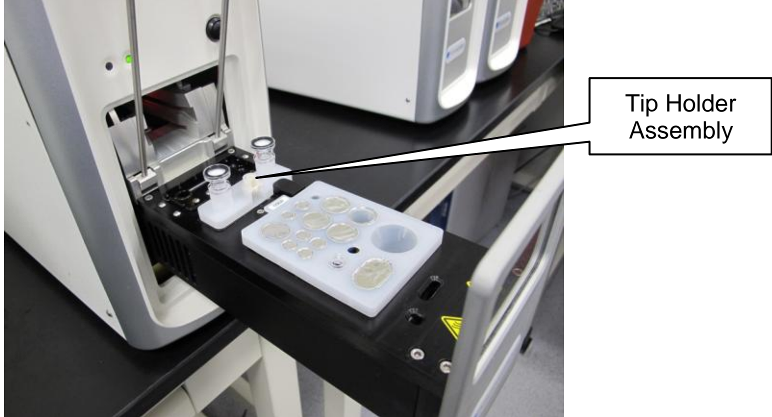
3. Loading the Tip Holder Assembly

   a) The Tip Holder Assembly is a plastic holder that contains two Pipette Tips and a rubber Tip Seal. Each Pipette Tip contains an O-ring on top.

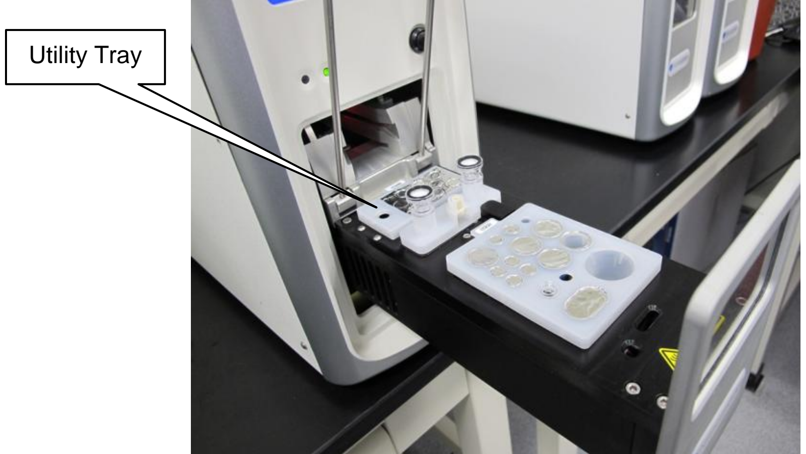


   b) Before using the Tip Holder Assembly, check the top of each Pipette Tip for the O-ring and confirm that the rubber Tip Seal is sitting straight and flush between the tips. If either is missing, replace with a new Tip Holder Assembly.

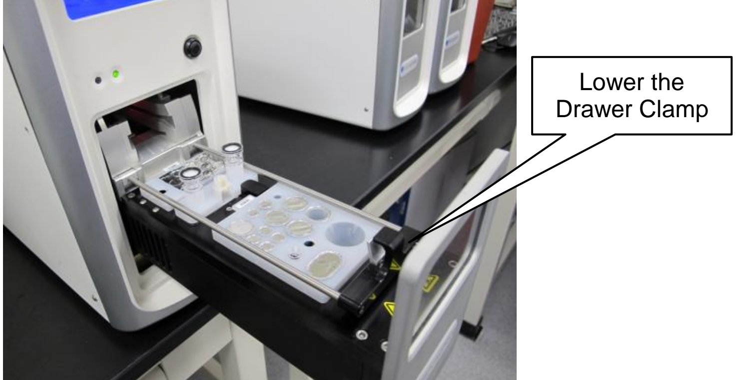
   c) Insert the Tip Holder Assembly into the Drawer Assembly. The image below shows a properly loaded Tip Holder Assembly. The Tip Holder Assembly can only be loaded in one location and orientation in the Drawer Assembly. For orientation, there are two holes on the deck of the Drawer Assembly that fit each Pipette Tip and the opening to the Tip Seal should face away from Processor *SP*.



4. Loading the Utility Tray
   a) (*optional*) Thaw the Utility Tray at room temperature for at least 10 minutes.
   b) (*optional*) After thawing, gently vortex (less than 5 seconds) the Utility Tray.
   c) (*optional*) Gently tap the tray on the bench to settle the reagents.
   d) Insert the Utility Tray into the Drawer Assembly. The image below shows a properly loaded Utility Tray. The Utility Tray can only be loaded in one location and orientation in the Drawer Assembly. When loaded properly, the tray sits flat.



   e) Lower and latch the Drawer Clamp over the Trays while supporting the Drawer with the opposite hand. The image below shows a closed Drawer Clamp over properly loaded trays and Tip Holder Assembly. The Drawer Clamp will latch onto the Drawer Assembly when closed properly, and the user will be unable to lift the Drawer Clamp without pressing in the silver latch.



5. Ordering a Test

a) All tests must be ordered through the Verigene Reader. No tests can be processed on the Verigene Processor *SP* without the user entering the Test Cartridge ID and Sample ID into the Verigene Reader.

i. Login to the Verigene Reader.

ii. To start a new Session, proceed to the next step (iii). To order a test in a previously created session, select the desired Session from the drop down 'SESSION' menu, then proceed to step (v).

Note: Up to 60 cartridges can be entered into a single session.

iii. From the Menu Bar, SESSION tab, select Start New Session where the Session Setup window will appear.

iv. Touch Session ID button and enter information by using the data entry keyboard. The Session ID can be any unique identifier in a format defined by the laboratory. The operator ID is automatically entered as the currently logged in 'user'.

v. Touch the Processing tab on the Navigation Bar at the bottom of the screen.

b) Enter the Test Cartridge ID by scanning the barcode using the barcode scanner attached to the Reader. The user may manually enter the Test Cartridge ID by selecting MENU and 'Enter Barcode' and then keying in the Test Cartridge ID number with the Reader's keyboard.

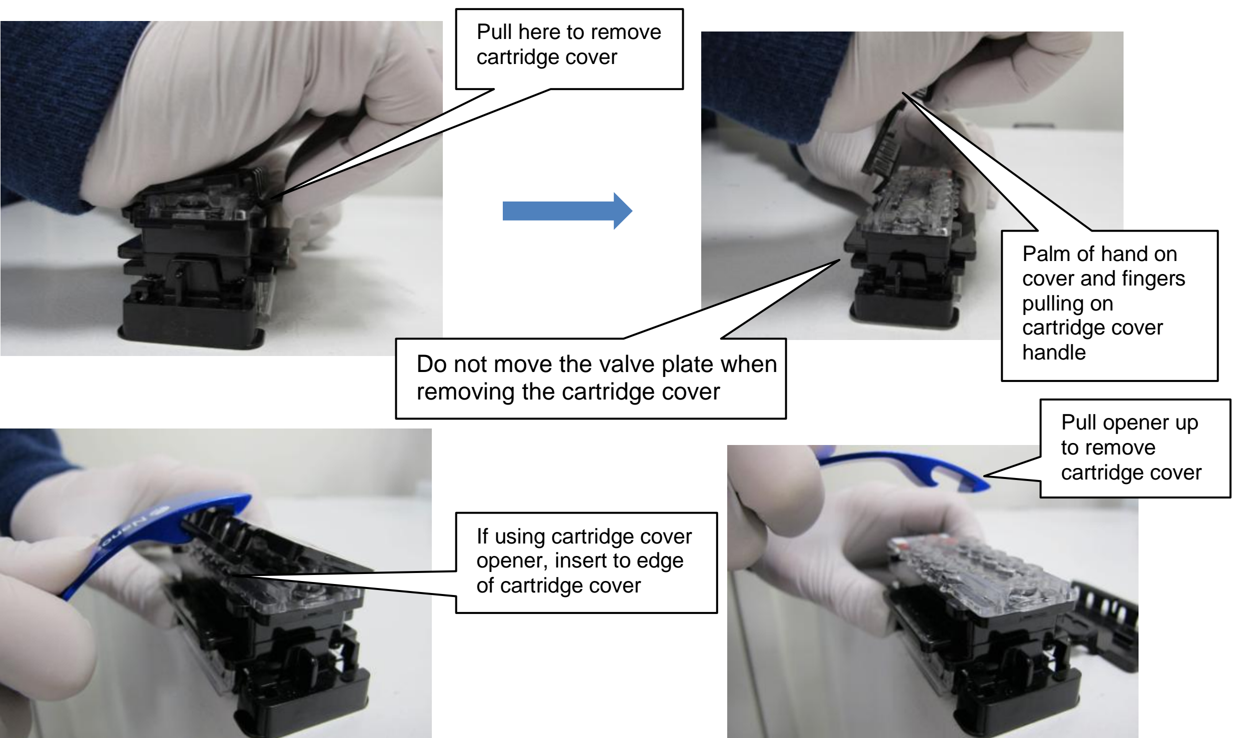
c) *(optional)* Scan the Test Cartridge Cover's 2D barcode using a barcode gun-style scanner to display the Test Cartridge's Reference Number, Expiration Date, and Lot Number on reports.

Note: The wand-style barcode scanner will not read 2D barcodes.

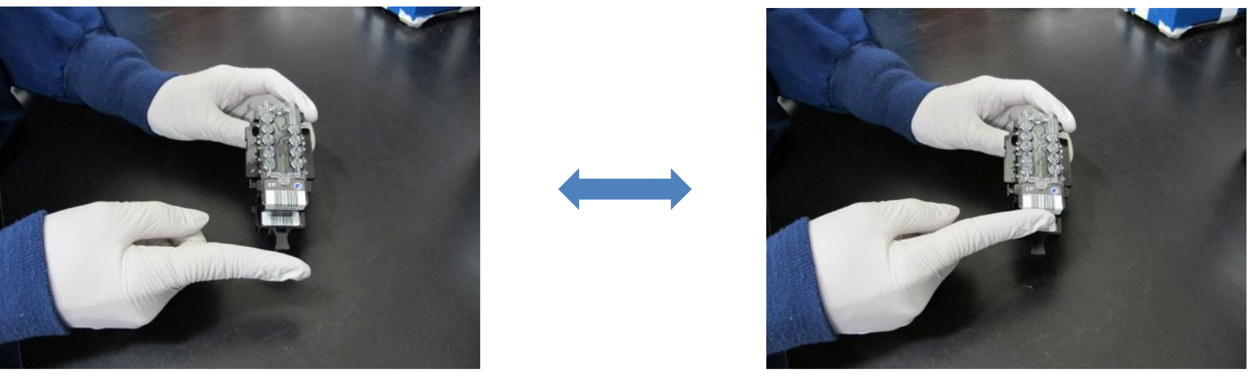
6. Loading a Test Cartridge

a) Hold the Test Cartridge by the handle with one hand, using the other hand apply pressure with the palm of the hand and remove the cartridge cover by bending the cover away and over the Reagent Pack edge. Ensure that the valve plate is not moved during cover removal (see illustration below).

Do not remove the Test Cartridge cover until immediately prior to inserting the Test Cartridge into the Processor *SP*.

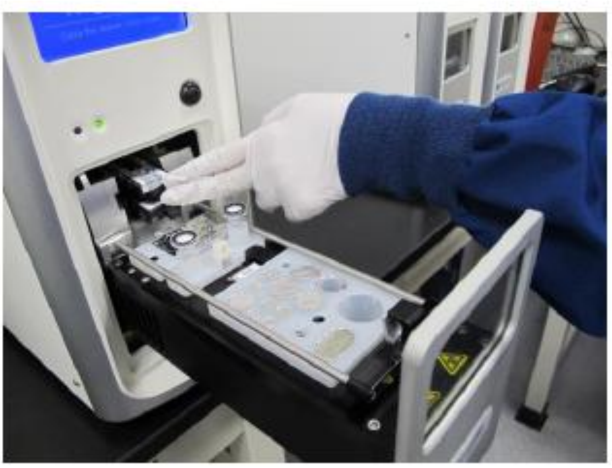


b) (*optional*) Settle the reagents in the cartridge before loading into the Processor *SP*. The optimal method for settling the reagents is to hold the reagent pack portion of the Test Cartridge on the side opposite the handle and tap the barcode end of the Cartridge with your index finger. When tapping the cartridge, allow the force of the tapping to move the cartridge and your right hand. The tapping is more effective when the cartridge is held in the air so that it moves slightly.



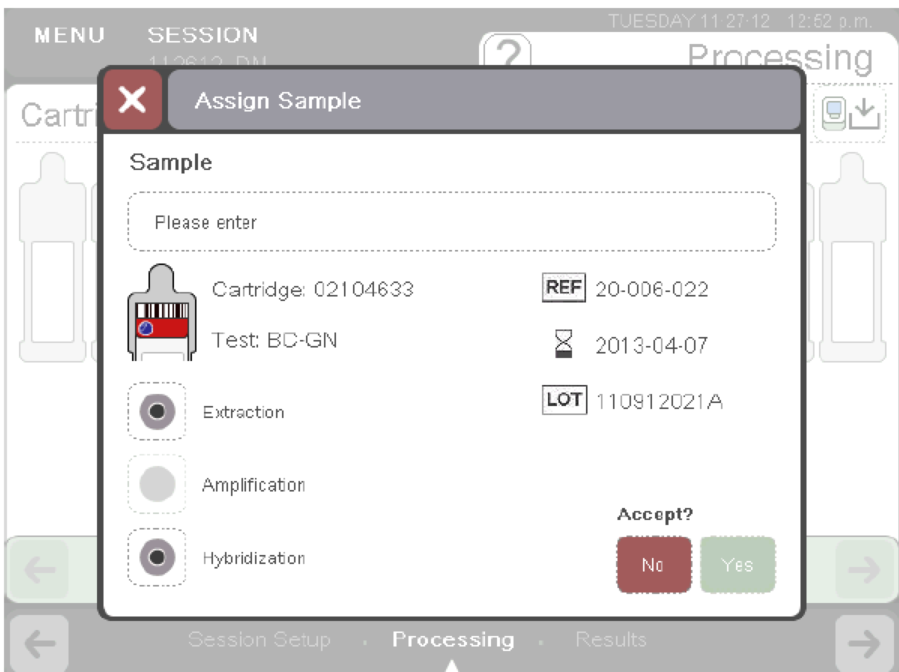
c) Insert the Test Cartridge into the Hybridization Module of the Processor *SP* until it reaches a stopping point. The image below shows the user loading a Test Cartridge into the Verigene Processor *SP*.

Note: If the Test Cartridge is not inserted properly, the Processor *SP* will display a message on the information screen when the user attempts to close the Drawer Assembly.



7. Loading the Sample

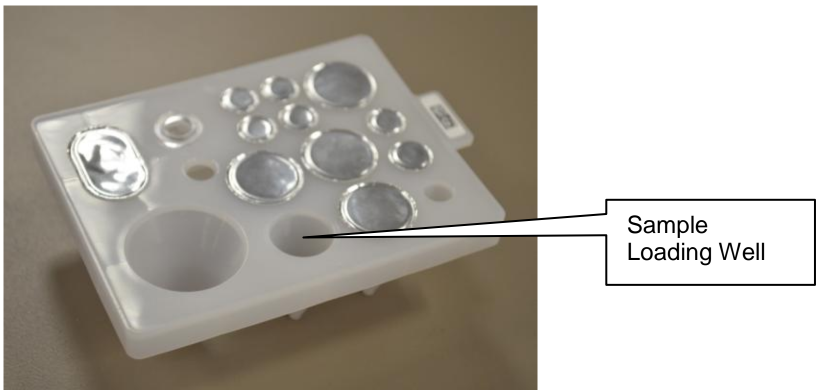
   a) Enter the Sample ID by scanning or manually enter the Sample ID using the Reader's touch-screen keyboard. Press Yes to confirm the Sample ID. Ensure the Extraction and Hybridization options are selected (see image below).



b) In the subsequent dialogue box, select or de-select the bacteria species or resistance marker from the list to activate or de-activate results reporting for those targets. Press Yes to confirm. The Verigene Reader will automatically default to the selected targets for the next test run.

Note: *Once a test run is started, results for de-selected targets cannot be retrieved.*

c) Gently vortex the gram-negative blood culture sample and pipette 700 µL of the gram-negative blood culture sample into the bottom of the Sample Loading Well in the Extraction Tray (refer to image below for Sample Loading Well location).



d) Close the Drawer Assembly by pressing the OPEN/CLOSE button on the Processor *SP*. The Processor will automatically verify that each consumable is properly loaded and begin sample processing.

e) Confirm countdown has started on the Processor *SP* display screen before leaving the area.

f) In order to set up additional tests on other Processor *SP* instruments follow the same procedure. To avoid contamination and sample mix-ups, set up one test at a time, change gloves after handling a sample, and decontaminate micro-pipettors and sample tubes between tests.

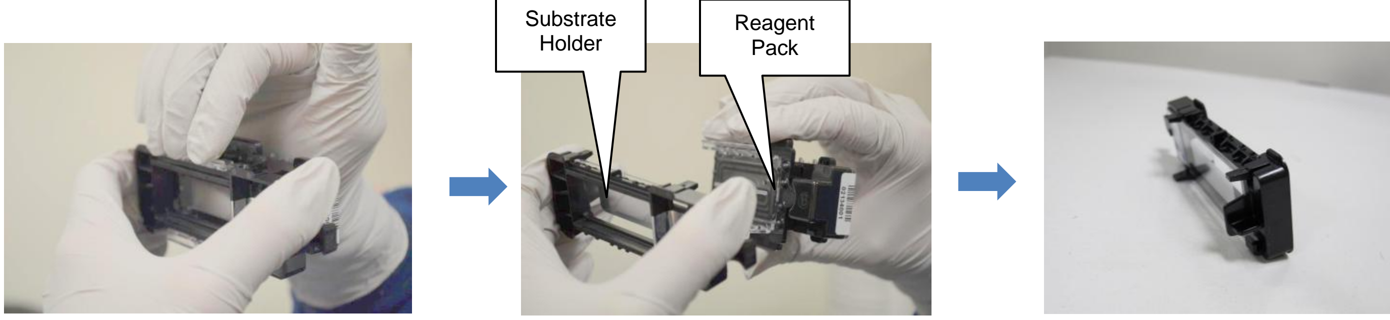
8. Upon completion of a test run

   a) The Verigene Reader will generate a ring to notify the user when the test is completed and the Processor *SP* will display a message indicating "Procedure Complete. Ready to Open Drawer." Remove the Test Cartridge from the Processor *SP* upon completion or within 12 hours of test completion.

   b) Open the Drawer Assembly by pressing the OPEN/CLOSE button.

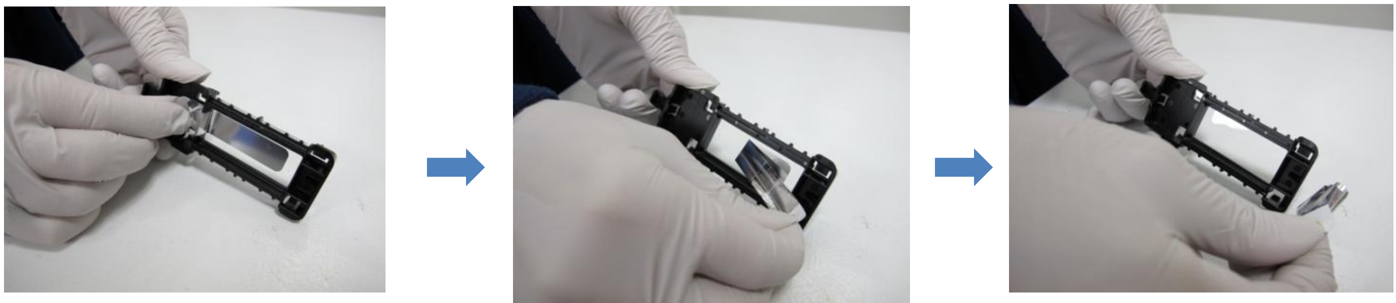
   c) Remove the Test Cartridge and immediately orient to its side.

   d) While keeping the Test Cartridge on its side, separate the Reagent Pack.



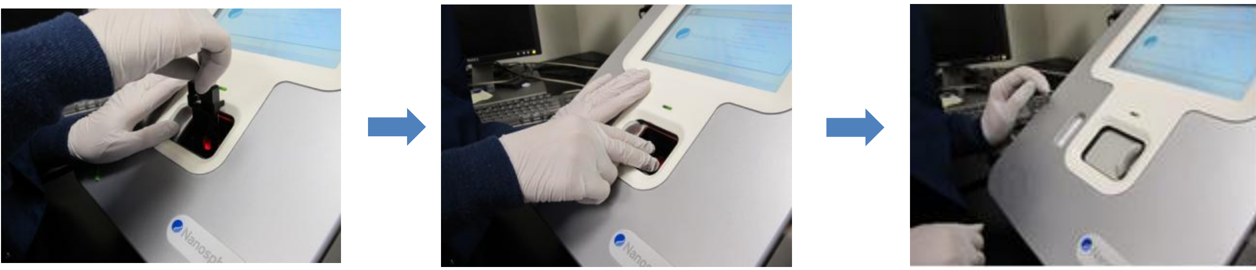
9. Analyzing results

   a) Remove the protective tape from the back of the slide in the Substrate Holder.



   b) Use the Reader's barcode scanner to read the barcode on the Substrate Holder. When the barcode is accepted, a prompt to load the Substrate Holder into the Reader will be displayed.

c) Immediately insert the Substrate Holder into the Reader.



d) Scanning the barcode ensures that the test result is associated with the correct sample. When the load substrate prompt occurs, it will only display for 20 seconds. The analysis will only start if the substrate is loaded during the animated prompt.

e) To properly insert the Substrate Holder into the Reader, hold the Substrate Holder by the handle with the barcode facing away from you. Next, insert the Substrate Holder into the Analysis Compartment. The compartment is designed to place the Holder in the correct position. Do not force the Substrate Holder in, but do insert it into the compartment as far as it will go comfortably. Close the door of the Analysis Compartment.

f) The analysis will automatically begin. A small camera icon will appear on the Reader to indicate that analysis has begun.

g) Once the analysis is completed by the Reader, the camera icon will be replaced with an upward facing arrow and the Reader rings.

h) Confirm that a result other than ‘No Call--NO GRID’ has been generated by touching the substrate icon for the test. A Substrate producing a ‘No Call--NO GRID’ result should be reanalyzed.

i) Once the scan is complete, dispose of the used Test Substrate.

10. Printing results

a) Touch the substrate icon in the Session’s Processing screen. A window displaying the results will open; touch the ‘Print’ option on this screen to print a Detail Report.

b) A Summary Report is available by moving to the Results screen of the Session on the bottom Navigation Bar; go to MENU then select ‘Print Summary’. The Summary Report will provide the results for all Tests processed within the current Session.

c) Detail Reports can also be viewed and printed from the Results window. First, select the desired Test from the list, go to MENU and then touch ‘Print Detail’.

INTERPRETATION OF RESULTS

BC-GN provides a qualitative result for the presence (“Detected”) or absence (“Not Detected”) of the **BC-GN** bacterial targets and antimicrobial resistance markers as listed in **Table 1**. The image analysis of the Test Substrate provides light signal intensities from the target-specific capture spots as well as the negative control, background, and imaging control spots. The mean signal intensity of a target is compared to the assay’s signal detection threshold to make a call. **Table 1** below lists the possible test results generated by **BC-GN**, representing identification of bacterial nucleic acid sequences/targets. ***Please note that BC-GN will only report resistance marker gene results when an organism is also detected by the assay.***

Table 1: Calls for Valid Results

Organism/Resistance Marker	***Target Gene***	***Test Result Reported as “Detected”***		
		Genus	*Species*	*Resistance Marker***
Acinetobacter spp	*rpsA*	Acinetobacter	-	-
Citrobacter spp.	*ompA/mrkC*	Citrobacter	-	-
Enterobacter spp.	*gyrB/metB*	Enterobacter	-	-
Proteus spp.	*atpD*	Proteus	-	-
Escherichia coli	*oppA*	-	E. coli *	-
Klebsiella pneumoniae	*yggE*	-	K. pneumoniae	-
Klebsiella oxytoca	*ompA*	-	K. oxytoca	-
Pseudomonas aeruginosa	*sodA*	-	P. aeruginosa	-
CTX-M	CTX-M	Any Acinetobacter, Citrobacter, Enterobacter, Proteus, E. coli, K. pneumoniae, K. oxytoca, and/or P. aeruginosa		CTX-M
KPC	KPC			KPC
NDM	NDM			NDM
VIM	VIM			VIM
IMP	IMP			IMP
OXA	OXA			OXA
All Analytes “Not Detected”	-	-	-	-

* Specimens containing *Shigella* spp. *and/*or *E. coli* will be reported as “E. coli Detected".

** A Not Detected result for any of the antimicrobial resistance markers does not indicate susceptibility, as resistance may occur by other mechanisms. Sub-culturing and AST testing is required in order to assign a resistant and/or susceptible phenotype to each isolate recovered from the blood culture sample.

Error calls related to an invalid test are listed in **Table 2,** together with the appropriate recourse, which should be taken by the user.

Table 2: Invalid Calls and Recourse

Call	***Reason***	***Recourse****
No Call – NO GRID	Reader unable to image Test Substrate	Ensure Test Substrate is seated properly in the Substrate Holder. Repeat image analysis by selecting 'Menu' and 'Enter Barcode' and then scanning the Substrate barcode. If the No-Call persists, repeat **BC-GN** from original blood culture specimen
No Call – INT CTL 1	INT CTL 1 Not Detected. Probable failure during the target hybridization part of the procedure only. This control does not require extraction to work properly.	Repeat **BC-GN**
No Call – INT CTL 2	INT CTL 2 Not Detected. Probable failure during extraction part of the procedure. This control requires extraction and hybridization to work properly.	
No Call – INT CTL	INT CTL 1 and INT CTL 2 Not Detected. Probable failure during the target hybridization and/or extraction portions of the procedure.	
No Call – VARIATION	Reader unable to obtain test result because of high variability in the target-specific signals.	
No Call – BKGD		
No Call – NEG CTL		
Processing Error	Pre-analytical error--Internal checks within the Processor *SP* detected an unexpected event.	Power cycle Processor *SP*, repeat **BC-GN** from original specimen.

QUALITY CONTROL

Quality control, as a component of an overall quality assurance program, consists of tests and procedures for monitoring and evaluating the analytical performance of a measurement system to ensure the reliability of test results.

A. Verigene System

The Verigene System uses a series of automated on-line quality measurements to monitor instrument functionality, software performance, fluidics, test conditions, reagent integrity, and procedural steps each time a test is performed. A series of automated on-line procedural checks guide the user through the testing process each time a test is performed. The **BC-GN** test barcode and sample information are linked upon entry into the Verigene Reader to help prevent misreporting of results.

B. Assay Controls

BC-GN is performed using single-use disposable reagent trays and cartridges, in which all reagents are prepackaged to prevent reagent dispensing errors. Several levels of controls are built into **BC-GN** to ensure that failures at any procedural step of **BC-GN** are identified during the procedure.

Internal Controls

BC-GN contains two sets of internal controls to ensure proper fluid control, hybridization, and signal detection. The first, referred to as INT CTL 1, is an internal processing control which detects the presence (hybridization and signal enhancement) of an artificial DNA oligonucleotide construct and mediator oligonucleotide contained within the sample buffer on the Extraction Tray. The second, referred to as INT CTL 2, verifies the presence of DNA from S*hewanella oneidensis*, which is in the **BC-GN** utility tray and added to the sample prior to the nucleic acid extraction step.

When detecting a negative sample, both the internal processing controls INT CTL 1 and INT CTL 2 must be present for a valid “Not Detected” call for all targets to be reported. If INT CTL 1 or INT CTL 2 is not detected, a “No Call – INT CTL 1” or a “No Call – INT CTL 2” result, respectively, is generated. If both INT CTL 1 and INT CTL 2 are not detected, a “NO CALL – INT CTL” result is generated. These internal controls are not utilized for the detection of positive samples.

External Controls

It is highly recommended that known blood culture specimens positive for each of the **BC-GN** panel organisms be tested routinely as defined by the user’s laboratory’s standard operating procedures on a rotating basis using 3-4 smaller groups of organisms, and/or under the following circumstances:

- Instrument installation, test validation, and when troubleshooting is necessary
- During performance verification for receipt of a new set/lot of consumables;
- When the integrity of consumables or the device is in question.

Frozen aliquots of blood cultures containing these organisms may be used for this purpose. When preparing QC material from a positive blood culture bottle, sterilize the bottle top by wiping with an alcohol wipe, invert the bottle ≥4 times to homogenize the specimen, draw fluid by using a 10 mL syringe (equipped preferably with a 16 gauge needle), and transfer to a secondary vessel. Vortex secondary vessel to homogenize specimen, dispense 1 mL aliquots into cryovials and store the aliquots at ≤-70 °C.

Regardless of the choice of quality control materials, all external quality control requirements and testing should be performed in conformance with local, state, and federal regulations or accreditation organizations as applicable and should follow the user’s laboratory standard quality control procedures.

TROUBLESHOOTING

Refer to the Troubleshooting section of the Verigene System User’s Manual.

LIMITATIONS

- A trained health care professional should interpret assay results together with the patient's medical history, clinical signs and symptoms, and the results of other diagnostic tests.
- In mixed cultures containing gram-negative bacteria and other organisms, **BC-GN** may not identify all the detectable organisms in the specimen.
- In rare instances for specimens with organisms carrying a resistance marker, **BC-GN** may not yield a positive result for the resistance marker when the organism(s) is detected.
- Isolation on solid media is needed for AST, to differentiate mixed growth with other organisms and to identify positive blood cultures yielding an all targets "Not Detected" result.
- The clinical study included evaluation of BACTEC™ Plus Aerobic/F and Standard Aerobic, BacT/ALERT® FA FAN® Aerobic, and VersaTREK® REDOX 1 and REDOX 1 EZ Draw® Aerobic bottles only. All other bottle types were evaluated analytically in the Universal Blood Culture Bottle Validation study (see Performance Characteristics section H).
- The detection of bacterial nucleic acid is dependent on proper specimen collection, handling, transport, storage, and preparation, including extraction. Failure to observe proper procedures in any of these steps could lead to incorrect results. False negative results may occur from improper specimen collection, handling or storage, technical error, sample mix-up, target concentration below the analytical sensitivity of the test, or below the concentration at bottle positivity, which might be caused by the growth of other organism(s).
- There is a risk of false negative or false positive results due to sequence variants in the bacterial targets of the assay.
- Detection of CTX-M, KPC, NDM, VIM, IMP, and/or OXA gene(s) in a specimen does not confirm that the resistant marker is associated with the organism(s) detected. Sub-culturing and subsequent testing of the isolated organism is necessary to definitively link antimicrobial resistance with a specific organism.
- Pediatric patient specimens were not differentiated from adult patient specimens in the clinical study; therefore, the performance characteristics of the assay with specimens obtained from pediatric patients have not been determined.
- Based on sequence homology analysis and analytical testing, a low likelihood of cross-reactivity exists between **BC-GN** probes which detect *Klebsiella oxytoca* and the nucleic acid sequence for *Klebsiella pneumoniae*. Therefore, on rare occasions, both a "K. oxytoca Detected" result and a "K. pneumoniae Detected" result may be obtained when *Klebsiella pneumoniae* is present in the specimen.
- *In silico* sequence analysis indicated sequence homology mismatches of *C. amalonaticus* that may yield false negative results, as demonstrated during **BC-GN** analytical testing, which resulted in detection of "Citrobacter" a total of 16 out of 18 tests (88.9%) for these organisms.
- **BC-GN** will not distinguish *Escherichia coli* from *Shigella* spp. including *S. dysenteriae, S. flexneri, S. boydii,* and *S. sonnei.*
- *Buttiauxella gaviniae* and *Enteric group 137 (ATCC BAA-69)* cross-react with **BC-GN** *Citrobacter* spp. probes, which will cause a false positive "Citrobacter Detected" result.
- *Escherichia albertii* strains cross-react with **BC-GN** E. coli probes, which will cause a false positive "E. coli Detected" result.
- *Kluyvera ascorbata, Raoultella ornithinolytica, Raoultella planticola,* and *Cedecea davisae* cross-react with **BC-GN** *Klebsiella oxytoca* probes, which will cause a false positive "K. oxytoca Detected" result.
- *Leminorella grimontii, Enterococcus raffinosus* and *Candida parapsilosis* cross-react with **BC-GN** CTX-M probes, which will cause a false positive "CTX-M Detected" result.
- **BC-GN** can only detect OXA types belonging to groups 23, 40, 48 and 58. OXA types from other groups cannot be detected by **BC-GN**. **BC-GN** will not detect OXA 51.
- Cultures containing mixed gram-positive and gram-negative organisms have not been evaluated with **BC-GN**.
- A combination of *in silico* analysis and wet testing was performed to demonstrate the ability of **BC-GN** to detect resistance marker genes coincident with the detection of a panel organism. However, wet testing was not performed on all bacterial species that are known to carry each resistance gene.

- Wet testing was not performed for all known resistance marker types and/or subtypes. Refer to **Table 19** for a listing of types that were evaluated by wet testing and/or by *in silico* analysis. Other types not listed in **Table 19** and/or in this limitations section have not been tested, therefore the ability of **BC-GN** to detect these types is unknown.
- **BC-GN** does not detect *Acinetobacter tartarogenes*, *Enterobacter gergoviae, Enterobacter kobei*, and *Enterobacter pyrinus*.
- False negative results for OXA may occur in certain *Acinetobacter radioresistens* strains that result as "Acinetobacter Detected" with **BC-GN**.
- Detection of IMP types 1, 4, 7, 8, 13, 15, 16, 18, 26, and 27 was demonstrated in the inclusivity study and/or clinical study. IMP types 2, 5, 6, 10, 11, 19, 20, 21, 24, 25, 28, 29, 30, 33, 37, 38, 40, 41 and 42 were not tested but *in silico* data shows that each has identical probe binding sites to types that have been tested, therefore, these types should be detected with a high degree of confidence. IMP types 3, 9, 12, 22, 32, 34, and 35 were not tested but are expected to be detected based on *in silico* data alone. IMP types 17, 23, 31, 36, and 39 neither were tested nor was there sequence information available to perform *in silico* analysis, therefore the ability of **BC-GN** to detect these types is unknown.
- Rare strains of *K. pneumoniae, Klebsiella variicola* and *Leclercia adecarboxylata* may cross-react with **BC-GN** *Enterobacter* spp. probes, which will cause a false positive "Enterobacter Detected" result.
- The performance of **BC-GN** for the detection of *A. baumannii* in the VersaTREK REDOX 1 EZ Draw /Aerobic bottle is unknown.
- Carbapenem resistance in the organisms detected by **BC-GN** can be due to mechanisms other than acquisition of the KPC (bla_{KPC}), OXA (bla_{OXA}), NDM (bla_{NDM}), VIM (bla_{VIM}), or IMP (bla_{IMP}) gene(s). Cephalosporin resistance in the organisms detected by **BC-GN** can be due to mechanisms other than acquisition of the CTX-M ($bla_{CTX\text{-}M}$) gene.
- Detection of a resistance marker does not always infer resistance.

WARNINGS AND PRECAUTIONS – GENERAL

- **BC-GN** is for *in vitro* diagnostic use.
- ***Caution: Federal law restricts this device to sale by or on the order of a physician or to a clinical laboratory***.
- Never use any tips, trays, tubes, or Test Cartridges which have been broken, cracked, punctured, previously used or anyway visibly damaged; using damaged material may lead to No Call or false results.
- Handle supplies, reagents, and kits with powder-free gloves at all times to avoid contamination and change gloves between removal of used disposables and loading of new disposables.
- Handle specimens carefully. Open one tube or specimen at a time to prevent specimen contamination.
- Biological specimens such as stool, tissues, body fluids, and blood of humans are potentially infectious. When handling and/or transporting human specimens, follow all applicable regulations mandated by local, state/provincial, and federal agencies for the handling/transport of etiologic agents.

WARNINGS AND PRECAUTIONS – INSTRUMENTS

A. General Instrument Safety
WARNING: Use this product only as specified in this document. Using this instrument in a manner not specified by Nanosphere may result in personal injury or damage to the instrument. Anyone who operates the instrument must have:
   - Received instructions in both general safety practices for laboratories and specific safety practices for the instrument.
   - Read and understood all applicable Material Safety Data Sheets (MSDS).

B. Electrical Shock Hazard
WARNING: Severe electrical shock can result from operating the instrument without its instrument covers or back panels in place. Do not remove instrument covers or panels. High-voltage contacts are exposed when instrument covers or panels are removed from the instrument. If service is required, outside the U.S. contact your local Nanosphere distributor.

WARNINGS AND PRECAUTIONS – REAGENTS AND TEST CARTRIDGES

A. Toxicity of Reagents

   - Exposure to chemicals sealed inside the Test Cartridge is hazardous in case of skin contact, respiratory inhalation or ingestion. Protective disposable gloves, laboratory coats, and eye protection should be worn when handling specimens, Extraction Trays, Utility Trays, and Test Cartridges.
   - See Material Safety Data Sheets (MSDS) for toxicity information. MSDS are available upon request from Nanosphere, Inc.

B. Waste Disposal

   - The Utility Tray contains a microorganism not known to cause disease in healthy humans (*Shewanella oneidensis).* Dispose of the Utility Tray in accordance with national, state, and local regulations.
   - The Extraction Tray contains residual nucleic acids, extraction reagents, and residual sample. It also contains a residual volume of the sample buffer that contains formamide, a teratogen. Dispose the Extraction Tray in accordance with national, state, and local regulations.
   - All of the Test Cartridge waste reagents, including the purified DNA, are contained within the Test Cartridge. There is a very small amount of residual formamide (≤1% v/v). Dispose the Test Cartridge in accordance with national, state, and local regulations.
   - Individual MSDSs are available for the Test Cartridge, Utility Tray and Extraction Tray at www.e-labeling.eu and at www.nanosphere.us.

EXPECTED VALUES

Prevalence

876 prospectively collected fresh and frozen blood culture specimens were obtained from twelve medium- to large-sized healthcare institutions geographically distributed across the United States. The number and percentage of positive cases (positivity rate) determined by the **BC-GN** test stratified by geographic region for each of the organisms and antimicrobial resistance markers detected by the **BC-GN** test are presented in **Table 3**. Overall, the **BC-GN** test detected at least one organism in 90.4% (792/876) and one resistance marker in 6.7% (59/876) of prospectively-collected specimens. In routine practice, prevalence rates may vary depending on the institution, geographical location, and patient population.

Table 3: Prevalence of Organisms Detected by **BC-GN** – Clinical Study Observations

		*US Geographic Region/Division**												
Organism	***Region***	*Midwest*						*South*		*Northeast*	*West*			***Total***
	Division	*West North Central*		*East North Central*				*South Atlantic*	*W. South Central*	*Middle Atlantic*	*Pacific*		*Mountain*	
	State	*NE*	*MN*	*MI*	*WI*	*IL*	*OH*	*MD*	*TX*	*NY*	*CA*	*WA*	*UT*	
	Total n	**23**	**36**	**108**	**74**	**64**	**67**	**90**	**145**	**88**	**26**	**67**	**88**	**876**
Acinetobacter	POS n	1	0	1	0	2	2	3	2	1	0	1	0	**13**
	% Prev.	4.3	-	0.9	-	3.1	3.0	3.3	1.4	1.1	-	1.5	-	**1.5**
Citrobacter	POS n	0	0	1	1	1	1	0	1	0	0	1	1	**7**
	% Prev.	-	-	0.9	1.4	1.6	1.5	-	0.7	-	-	1.5	1.1	**0.8**
Enterobacter	POS n	2	2	5	7	2	6	11	11	4	1	5	11	**67**
	% Prev.	8.7	5.6	4.6	9.5	3.1	9.0	12.2	7.6	4.6	3.8	7.5	12.5	**7.6**
Proteus	POS n	2	1	6	3	5	0	1	8	3	0	1	2	**32**
	% Prev.	8.7	2.8	5.6	4.1	7.8	-	1.1	5.5	3.4	-	1.5	2.3	**3.7**
E. coli	POS n	11	23	60	28	36	23	23	85	52	13	28	47	**429**
	% Prev.	47.8	63.9	55.6	37.8	56.3	34.3	25.6	58.6	59.1	50.0	41.8	53.4	**49.0**
Klebsiella pneumoniae	POS n	6	0	18	16	8	13	21	20	8	3	4	11	**128**
	% Prev.	26.1	-	16.7	21.6	12.5	19.4	23.3	13.8	9.1	11.5	6.0	12.5	**14.6**
Klebsiella oxytoca	POS n	0	3	3	4	2	2	7	2	3	0	5	6	**37**
	% Prev.	-	8.3	2.8	5.4	3.1	3.0	7.8	1.4	3.4	-	7.5	6.8	**4.2**
Pseudomonas aeruginosa	POS n	0	7	6	10	3	6	9	9	9	5	11	3	**78**
	% Prev.	-	19.4	5.6	13.5	4.7	9.0	10.0	6.2	10.2	19.2	16.4	3.4	**8.9**
Resistance Marker (linked)	**Total n**	**21**	**35**	**98**	**67**	**56**	**52**	**73**	**136**	**78**	**22**	**55**	**80**	**773**
NDM	POS n	0	0	0	1	0	0	0	0	0	0	0	0	**1**
	% Prev.	-	-	-	1.5	-	-	-	-	-	-	-	-	**0.1**
KPC	POS n	0	0	0	0	1	1	1	0	0	0	0	0	**3**
	% Prev.	-	-	-	0	1.8	1.9	1.4	-	-	-	-	-	**0.4**
CTX-M	POS n	0	3	7	4	5	1	8	9	0	3	4	6	**50**
	% Prev.	-	8.6	7.1	6.0	8.9	1.9	11.0	6.6	-	13.6	7.3	7.5	**6.5**
VIM	POS n	0	0	0	0	0	0	0	0	0	0	0	0	**0**
	% Prev.	-	-	-	-	-	-	-	-	-	-	-	-	**-**
IMP	POS n	0	0	0	0	0	0	0	0	0	0	0	0	**0**
	% Prev.	-	-	-	-	-	-	-	-	-	-	-	-	**-**
OXA	POS n	0	0	0	0	1	1	2	0	1	0	0	0	**5**
	% Prev.	-	-	-	-	1.8	1.9	2.7	-	1.3	-	-	-	**0.6**

(1) *Geographic Areas Reference Manual* (US Census Bureau). Chapter 6 https://www.census.gov/geo/reference/pdfs/GARM/Ch6GARM.pdf.Webpage last revised: 3/4/2013

PERFORMANCE CHARACTERISTICS

The results of the analytical and clinical studies conducted to establish the performance characteristics of **BC-GN** are provided below.

A. Clinical Performance

A method comparison study was conducted at multiple external clinical study sites to evaluate the performance of **BC-GN** by comparing **BC-GN** bacterial test results to reference culture, followed by bacterial biochemical identification, and for bacterial resistance markers, PCR amplification followed by confirmatory bi-directional sequencing. Subjects included individuals whose routine care called for blood culture testing and whose blood culture specimens were positive for microbial growth and identified by Gram stain as gram-negative.

There were 1434 evaluable specimens enrolled in the clinical trial; 62 specimens had an initial **BC-GN** "No Call" rate of 4.3% (62/1434 specimens) and 11 specimens had an initial Pre-Analytical Error (Pre-AE) rate of 0.8% (11/1434 tests run) for a total initial valid test rate of 94.9%. Of the 62 initial No Calls, 41 yielded a valid test result upon retesting and of the 11 initial Pre-AEs, 10 yielded a valid test result upon repeat and one (1) was classified as a final Pre-AE. The final "No Call" rate was 1.5% (21/1434 specimens) and the final Pre-Analysis Error rate was 0.8% (12/1507 tests run) for a total final valid test rate of 97.7%. The twenty-one (21) specimens which yielded a final "No Call" result and the one (1) specimen which yielded a final Pre-AE (22 specimens in total), were not included in the valid dataset utilized in the comparative test result data analysis. Therefore, 98.5% (1412/1434) of the valid specimens were analyzed in this clinical evaluation to establish clinical performance of the test; 604 of which were prospectively-collected fresh specimens, 272 of which were prospectively-collected frozen specimens, 239 of which were selected frozen specimens, and 297 of which were simulated frozen specimens.

The clinical performance of **BC-GN** is summarized below in **Table 4** for the four genus level bacterial targets (n=1412) and in **Table 5** for the four species-level bacterial targets (n=1412). Bacterial **BC-GN** test results obtained during the study were compared with reference results obtained from culture/conventional and automated phenotypic biochemical identification techniques.

Of the 1412 specimens analyzed, 146 specimens contained organisms not detected by **BC-GN** is summarized below in **Table 6**. Because the **BC-GN** reporting algorithm links the reporting of any resistance marker with the detection of one or more bacterial targets, the clinical performance of **BC-GN** for the 6 resistance markers was determined with the 1266 specimen dataset and summarized in **Table 7**. Resistance marker **BC-GN** test results obtained during the study were compared with reference results obtained from resistance marker-specific PCR amplification and confirmatory bi-directional sequencing.

In total, there were 22 mixed specimens that were detected by **BC-GN**, and 35 mixed specimens detected by the reference culture methods. **Table 8** lists the distinct mixed specimen combinations detected by **BC-GN** in the clinical study and **Table 9** lists the additional distinct mixed specimen combinations detected by the reference/comparator methods, but not detected by **BC-GN**.

Table 10 contains additional genus/group-level specific **BC-GN** performance data stratified by individual species within each genus; i.e.; *Acinetobacter* spp., *Citrobacter* spp., *Enterobacter* spp., and *Proteus* spp.

The performance of **BC-GN** for the detection of resistance markers, as described in **Table 7**, is further detailed by organism (as detected by the reference method) in **Table 11** and **Table 12,** for "CTX-M, OXA**,** KPC" and "IMP, VIM, NDM", respectively. **Table 13** provides a summary of the organisms in the clinical study that were found to contain single and dual resistance markers.

Table 4: Summary of Clinical Test Performance (n=1412) - Compared to Reference Methods (Culture and Conventional Biochemical and Automated Phenotypic Identification)

Genus-Level Targets *Acinetobacter, Citrobacter, Enterobacter,* and *Proteus*

Target	Specimen Type		n	% Agreement (95% CI) Positive	% Agreement (95% CI) Negative
***Acinetobacter* spp.**	*Prospective*	Fresh	604	100% 12/12 (73.5-100)	100% 592/592 (99.4-100)
	Prospective	Frozen	272	50% 1/2 (1.3-98.7)	100% 270/270 (98.6-100)
	Selected	Frozen	239	100% 15/15 (78.2-100)	99.6% 223/224 (97.6-100)
	Simulated	Frozen	297	100% 27/27 (87.2-100)	100% 270/270 (98.6-100)
	All		***1412***	***98.2% 55/56*** [a] ***(90.5-100)***	***99.9% 1355/1356*** [b] ***(99.6-100)***

Target	Specimen Type		n	% Agreement (95% CI) Positive	% Agreement (95% CI) Negative
***Citrobacter* spp.**	*Prospective*	Fresh	604	100% 5/5 (47.8-100)	99.8% 598/599 (99.1-100)
	Prospective	Frozen	272	100% 1/1 (2.5-100)	100% 271/271 (98.7-100)
	Selected	Frozen	239	100% 13/13 (75.3-100)	100% 226//226 (98.4-100)
	Simulated	Frozen	297	100% 30/30 (88.4-100)	100% 267/267 (98.6-100)
	All		***1412***	***100% 49/49 (92.8-100)***	***99.9% 1362/1363*** [c] ***(99.6-100)***

Target	Specimen Type		n	% Agreement (95% CI) Positive	% Agreement (95% CI) Negative
***Enterobacter* spp.**	*Prospective*	Fresh	604	95.6% 43/45 (84.9-99.5)	100% 559/559 (99.3-100)
	Prospective	Frozen	272	95.2% 20/21 (76.2-99.9)	98.4% 247/251 (96.0-99.6)
	Selected	Frozen	239	100% 29/29 (88.1-100)	98.1% 206/210 (95.2-99.5)
	Simulated	Frozen	297	100% 28/28 (87.7-100)	100% 269/269 (98.6-100)
	All		***1412***	***97.6% 120/123*** [d] ***(93.0-99.5)***	***99.4% 1281/1289*** [e] ***(98.8-99.7)***

Target	Specimen Type		n	% Agreement (95% CI) Positive	% Agreement (95% CI) Negative
***Proteus* spp.**	*Prospective*	Fresh	604	100% 20/20 (83.2-100)	100% 584/584 (99.4-100)
	Prospective	Frozen	272	100% 12/12 (73.5-100)	100% 260/260 (98.6-100)
	Selected	Frozen	239	100% 24/24 (85.8-100)	99.5% 214/215 (97.4-100)
	Simulated	Frozen	297	100% 2/2 (15.8-100)	100% 295/295 (98.8-100)
	All		***1412***	***100% 58/58 (93.8-100)***	***99.9% 1353/1354*** [f] ***(99.6-100)***

	No.	***Identified by BC-GN test as:***	***Identified by Reference Method(s) as:***	***PCR Amp/BD Sequencing Results (if applicable)***
a.	1	"Not Detected"	*A. baumannii*	Negative for *Acinetobacter* spp.
b.	1	"Acinetobacter"	*Enterococcus* spp.	Negative for *Acinetobacter* spp.
c.	1	"Citrobacter"	*S. marcescens*	*C. freundii* (Low quality score ID) and *S. marcescens*
d.	1	"K. oxytoca"	*E. cloacae* complex and *K. oxytoca*	*E. cloacae/E. aerogenes*
	1	"K. oxytoca" "K. pneumoniae"	*K. oxytoca*, *K. pneumoniae* and *E. cloacae* complex	*E. cloacae/E. aerogenes* and *K. oxytoca*
	1	"E. coli"	*E. cloacae*	*E. coli*
e.	3	"K. pneumoniae" "Enterobacter"	*K. pneumoniae*	Unable to differentiate: Similar match to both *K. variicola* and *K. pneumoniae*
		"K. pneumoniae" "Enterobacter"	*K. pneumoniae*	Unable to differentiate: Similar match to both *K. variicola* and *K. pneumoniae*
		"K. pneumoniae" "Enterobacter"	*K. pneumoniae*	Unable to differentiate: Similar match to both *K. variicola* and *K. pneumoniae*
	1	"Enterobacter"	*E. coli*	*E. cloacae*
	1	"Enterobacter"	*K. pneumoniae*	*K. variicola*
f.	1	"Proteus"	No growth by culture	N/A

Table 5: Summary of Clinical Test Performance (n=1412) - Compared to Reference Methods (Culture and Conventional Biochemical and Automated Phenotypic Identification)

Species-Level Targets *E. coli, K. pneumonia, K. oxytoca,* and *P. aeruginosa*

Organism	Specimen Type		n	% Agreement (95% CI) Positive	% Agreement (95% CI) Negative
Escherichia coli	*Prospective*	Fresh	604	100% 283/283 (98.7-100)	99.1% 318/321 (97.3-99.8)
	Prospective	Frozen	272	99.3% 142/143 (96.2-100)	99.2% 128/129 (95.8-100)
	Selected	Frozen	239	100% 42/42 (91.6-100)	99.5% 196/197 (97.2-100)
	Simulated	Frozen	297	100% 50/50 (92.9-100)	100% 247/247 (98.5-100)
	All		***1412***	***99.8% 517/518 [g] (98.9-100)***	***99.4% 889/894 [h] (98.7-99.8)***
Pseudomonas aeruginosa	*Prospective*	Fresh	604	97.1% 67/69 (89.9-99.7)	100% 535/535 (99.3-100)
	Prospective	Frozen	272	91.7% 11/12 (61.5-99.8)	100% 260/260 (98.6-100)
	Selected	Frozen	239	100% 19/19 (82.4-100)	100% 220/220 (98.3-100)
	Simulated	Frozen	297	100% 27/27 (87.2-100)	100% 270/270 (98.6-100)
	All		***1412***	***97.6% 124/127 [l] (93.3-99.5)***	***100% 1285/1285 (99.7-100)***
Klebsiella oxytoca	*Prospective*	Fresh	604	95.7% 22/23 (78.1-99.9)	98.2% 576/581 (95.9-99.4)
	Prospective	Frozen	272	100% 9/9 (66.4-100)	99.6% 262/263 (97.9-100)
	Selected	Frozen	239	92.6% 25/27 (75.7-99.1)	100% 212/212 (98.3-100)
	Simulated	Frozen	297	60.0% 3/5 (14.7-94.7)	100% 292/292 (98.7-100)
	All		***1412***	***92.2% 59/64 [j] (82.7-97.4)***	***99.6% 1342/1348 [k] (99.0-99.8)***
Klebsiella pneumoniae	*Prospective*	Fresh	604	88.0% 88/100 (80.0-93.6)	100% 504/504 (99.3-100)
	Prospective	Frozen	272	87.0% 40/46 (73.7-95.1)	100% 226/226 (98.4-100)
	Selected	Frozen	239	94.7% 36/38 (82.3-99.4)	100% 201/201 (98.2-100)
	Simulated		297	99.2% 121/122 (95.5-100)	100% 175/175 (97.9-100)
	All		***1412***	***93.1% 285/306 [i] (89.7-95.7)***	***100% 1106/1106 (99.7-100)***

	No.	Identified by BC-GN test as:	Identified by Reference Method(s) as:	PCR Amp/BD Sequencing Results (if applicable)
g.	1	"Enterobacter"	*E. coli*	*E. cloacae*
h.	1	"E. coli"	*E. cloacae*	*E. coli*
	1	"K. pneumoniae" "E. coli"	*K. pneumoniae*	*Shigella* spp.
	1	"E. coli"	*K. oxytoca*	*E. coli*
	1	"K. oxytoca" "E. coli"	*K. oxytoca*	*K. oxytoca* and *E. coli*
	1	"Enterobacter" "E. coli"	*E. cloacae* complex	*Shigella* spp.
i.	17	"Not Detected"	*K. pneumoniae*	Unable to differentiate: Similar match to both *K. variicola* and *K. pneumoniae*
		"Not Detected"	*K. pneumoniae*	*K. variicola*
		"Not Detected"	*K. pneumoniae*	*K. variicola*
		"Not Detected"	*K. pneumoniae*	*K. variicola*
		"Not Detected"	*K. pneumoniae*	*K. variicola*
		"Not Detected"	*K. pneumoniae*	*Klebsiella* spp.
		"Not Detected"	*K. pneumoniae*	Unable to differentiate: Similar match to both *K. variicola* and *K. pneumoniae*
		"Not Detected"	*K. pneumoniae*	*K. variicola*
		"Not Detected"	*K. pneumoniae*	*K. variicola*
		"Not Detected"	*K. pneumoniae*	*K. variicola*
		"Not Detected"	*K. pneumoniae*	*K. variicola*
		"Not Detected"	*K. pneumoniae*	*K. variicola*
		"Not Detected"	*K. pneumoniae*	*K. variicola*
		"Not Detected"	*K. pneumoniae*	Unable to differentiate: Similar match to both *K. variicola* and *K. pneumoniae*
		"Not Detected"	*K. pneumoniae*	*K. variicola*
		"Not Detected"	*K. pneumoniae*	*K. variicola*
		"Not Detected"	*K. pneumoniae*	*K. variicola*
	1	"E. coli"	*E. coli*, *K. pneumoniae* and *S. dysgalactiae*	*K. pneumoniae* (*E. coli* not tested)
	1	"KPC"	*K. pneumoniae* and KPC	*K. pneumoniae*
	1	"Enterobacter"	*K. pneumoniae*	*K. variicola*
	1	"E. coli"	*K. oxytoca*	*Klebsiella* spp.
j.	1	"Not Detected"	*K. oxytoca*	
	1	"Not Detected"	*K. oxytoca*	*K. variicola*
	1	"E. coli" "CTX-M"	*K. oxytoca, E. coli,* CTX-M Gp. 1	*K. oxytoca* and *E. coli*
	1	"E. coli"	*K. oxytoca* and *E. coli*	*K. oxytoca* and *E. coli*
	1	"KPC"	*K. oxytoca* and KPC	*K. oxytoca*
k.	5	"K. oxytoca"	*R. planticola*	*R. planticola*
		"K. oxytoca"	*R. planticola*	*R. planticola*
		"K. oxytoca"	*R. planticola*	*R. planticola*
		"K. oxytoca"	*R. planticola*	*R. planticola*
		"K. oxytoca"	*R. planticola*	*R. planticola*
	1	"K. pneumoniae" "K. oxytoca"	*K. pneumoniae*	*K. pneumoniae*
l.	1	"K. pneumoniae" "CTX-M"	*P. aeruginosa*, *K. pneumoniae* and CTX-M	*P. aeruginosa* (*K. pneumoniae* not tested)
	1	"K. pneumoniae"	*P. aeruginosa* and *K. pneumoniae*	*P. aeruginosa*
	1	"E. coli"	*P. aeruginosa* and *E. coli*	*P. aeruginosa*

Table 6: Summary of **BC-GN** "Not Detected" Organisms (n=146) – Identification by Reference Method

Specimen Type		***n=146***	***Organism Detected by Reference Method***
Prospective	Fresh	67	*C.gleum* (1); *Corynebacterium* spp.& *S. paucimobilis* (1); *D. acidovorans* & *S. maltophilia* (1); *E. faecium* & *S. maltophilia* (1); *K. pneumoniae* (10); *Moraxella* group (2); *M. morganii* (2); *Pandoraea* spp. (1); *P. agglomerans* (1); *P. canis* (2); *P. shigelloides* (1); *P. stuartii* (1) *P. citronellolis* (1); *P. fluorescens* (1); *P. fluorescens/putida* (1); *P. mendocina* (1); *P. monteilii* (1); *P. putida* (2); *S. putida* & *S. hominis* (1); *P. stutzeri* (2); *R. aquatilis* (1); *R. pickettii* (1); *Salmonella* group (2); *S. liquefaciens* group (5); *S. marcescens* (10); *S. marcescens* & *A. xylosoxidans* (1); *S. marcescens* & *S. hominis* (1); *S. paucimobilis* (3); *S. maltophilia* (6); *Streptococcus* spp. (1); No growth (2)
	Frozen	36	*A. baumannii* complex (1); *B. vesicularis* (1); *B. cepacia* (1); *B. gladioli* (1); *C. pauculus* & *S. maltophilia* (1); *E. meningoseptica* (1); *H. influenzeae* (1); *K. pneumoniae* (6); *Moraxella* group (1); *M. nonliquefaciens* (1); *M. morganii* (2); *P. canis* (2); *P. putida* (1); *S. enterica* (1); *Salmonella* group (2); *S. marcescens* (6); *S. odorifera* & *Pantoea* spp. *(1); S. paucimobilis* (2); *S. maltophilia* (4)
Selected	Frozen	33	*E.faecium* (1); *E. faecalis* (1); *K. oxytoca* (1); *K. pneumoniae* (1); *S. marcescens* (26); *S. maltophilia* (1); *S. aureus* (1); No growth (1)
Simulated	Frozen	10	*K. oxytoca* (1); *K. pneumoniae* (1); *M. morganii* (4); *P. putida* (1); *S. marcescens* (2); *S. putrefaciens* (1)

Table 7: Summary of Clinical Test Performance (n=1266) - Compared to Reference Methods (PCR Amplification/Bi-Directional Sequencing)

Resistance Marker Targets CTX-M, OXA, KPC, VIM, NDM, and IMP

	Specimen Type		***n***	***% Agreement (95% CI)***			***Specimen Type***		***n***	***% Agreement (95% CI)***	
				Positive	***Negative***					***Positive***	***Negative***
OXA	*Prospective*	Fresh	537	100 % 5/5 (47.8-100)	100% 532/532 (99.3-100)	CTX-M	*Prospective*	Fresh	537	97.5% 39/40 (86.8-99.9)	100% 497/497 (99.3-100)
		Frozen	236	-	100% 236/236 (98.5-100)			Frozen	236	91.2% 11/12 (61.5-99.8)	100% 224/224 (98.4-100)
	Selected	Frozen	206	50.0% 2/4 (6.8-93.2)	100% 202/202 (98.2-100)		*Selected*	Frozen	206	100% 3/3 (29.2-100)	100% 203/203 (98.2-100)
	Simulated	Frozen	287	98.3% 54/55 (90.8-100)	99.6% 231/232 (97.6-100)		*Simulated*	Frozen	287	100% 98/98 (96.3-100)	99.5% 188/189 (97.1-100)
	All		***1266***	***95.3% 61/64 [m] (86.9-99.0)***	***99.9% 1201/1202 [n] (99.5-100)***		***All***		***1266***	***98.7% 151/153 [k] (95.4-99.8)***	***99.9% 1112/1113 [l] (99.5-100)***
KPC	*Prospective*	Fresh	537	100% 2/2 (15.8-100)	100% 535/535 (99.3-100)	NDM	*Prospective*	Fresh	537	100% 1/1 (2.5-100)	100% 536/536 (99.3-100)
		Frozen	236	100% 1/1 (2.5-100)	100% 235/235 (98.5-100)			Frozen	236	-	100% 236/236 (98.5-100)
	Selected	Frozen	206	-	100% 206/206 (98.2-100)		*Selected*	Frozen	206	-	100% 206/206 (98.2-100)
	Simulated	Frozen	287	100% 48/48 (92.6-100)	100% 239/239 (98.5-100)		*Simulated*	Frozen	287	100% 40/40 (91.2-100)	100% 247/247 (98.5-100)
	All		***1266***	***100% 51/51 (93.1-100)***	***100% 1215/1215 (99.7-100)***		***All***		***1266***	***100% 41/41 (91.4-100)***	***100% 1225/1225 (99.7-100)***
IMP	*Prospective*	Fresh	537	-	100% 537/537 (99.3-100)	VIM	*Prospective*	Fresh	537	-	100% 537/537 (99.3-100)
		Frozen	236	-	100% 236/236 (98.5-100)			Frozen	236	-	100% 236/236 (98.5-100)
	Selected	Frozen	206	-	100% 206/206 (98. 2-100)		*Selected*	Frozen	206	-	100% 206/206 (98.2-100)
	Simulated	Frozen	287	100% 48/48 (92.6-100)	100% 239/239 (98.5-100)		*Simulated*	Frozen	287	100% 41/41 (91.4-100)	100% 246/246 (98.5-100)
	All		***1266***	***100% 48/48 (92.6-100)***	***100% 1218/1218 (99.7-100)***		***All***		***1266***	***100% 41/41 (91.4-100)***	***100% 1225/1225 (99.7-100)***

	No.	***Identified by BC-GN test as:***	***Identified by Reference Method(s) as:***
k.	1	"E. coli"	*Escherichia coli* and CTX-M Gp1
	1	"E. coli" and "Enterobacter"	*Escherichia coli* and CTX-M Gp1
l.	1	"Citrobacter", "CTX-M" and "OXA"	*C. braakii* and OXA Gp48
m.	2	"Acinetobacter"	*Acinetobacter baumannii* and OXA Gp23
	1	"Acinetobacter"	*Acinetobacter radioresistans* and OXA Gp23
n.	1	"Acinetobacter" and "OXA"	*Acinetobacter baumannii*
o.	One FN "Acinetobacter" and one FP "Acinetobacter" and "OXA" were consecutively tested specimens and may be due to a sample mix-up.		

Table 8: Clinical Mixed Specimen Combinations Detected by **BC-GN**

Multiple Organism Combinations Detected by BC-GN [1]				***Reference Test***			***Sample ID(s)***
Organism 1	*Organism 2*	*Organism 3*	*Resistance Marker*	*Total Specimens*	*Discrepant Specimens*	Discrepant Analyte(s)[1]	
Escherichia coli	*Klebsiella pneumoniae*	*Acinetobacter* spp.	NONE	1	0	-	07072
Acinetobacter spp.	*Klebsiella pneumoniae*		NONE	1	0	-	06097
Acinetobacter spp.	*Escherichia coli*		OXA	1	0	-	99038
Acinetobacter spp.	*Enterobacter* spp.		NONE	1	0	-	99227
Enterobacter spp.	*Escherichia coli*		NONE	2	2	*Escherichia coli,* CTX-M	15074 11065
Enterobacter spp.	*Klebsiella pneumoniae*		NONE	3	2	*E. asburaie Enterobacter* spp.	06062 12077 17081
Enterobacter spp.	*Klebsiella pneumoniae*		IMP/VIM	1	0	-	99472
Enterobacter spp.	*Klebsiella oxytoca*		NONE	1	0	-	09098
Enterobacter spp.	*Klebsiella pneumoniae*		NONE	1	1	*Enterobacter* spp.	14017
Escherichia coli	*Proteus* spp.		NONE	3	0	-	12041 13035 15071
Escherichia coli	*Klebsiella oxytoca*		NONE	2	1	*Escherichia coli*	99163 07034
Escherichia coli	*Klebsiella pneumoniae*		NONE	3	1	*Escherichia coli*	08098 10023 99243
Klebsiella oxytoca	*Klebsiella pneumoniae*		NONE	2	2	*K. oxytoca, Enterobacter cloacae* complex	08105 09011
TOTAL				***22***	***9***		

[1] Defined as an analyte that was detected by the **BC-GN** test, but not detected by the reference methods.

Table 9: Clinical Mixed Specimen Combinations Detected by Reference Methods

Multiple Organism Combinations by Reference Test[1]				***Detected by BC-GN***			*Sample ID(s)*
Organism 1	*Organism 2*	*Organism 3*	*Resistance Marker*	*Total Specimens*	*Discrepant Specimens*	*Discrepant Analyte(s)*[1]	
A. baumannii complex	*Enterococcus spp*	*Escherichia coli*	OXA	1	0	-	99038
Enterobacter cloacae complex	*Klebsiella oxytoca*	*Klebsiella pneumoniae*	NONE	1	1	*Enterobacter cloacae* complex	09011
Escherichia coli	*Klebsiella pneumoniae*	*Streptococcus dysgalactiae*	NONE	1	1	*Klebsiella pneumoniae*	09050
Escherichia coli	*Proteus mirabilis*	*Sphingomonas paucimobilis*	NONE	1	0	-	13035
Klebsiella oxytoca	*Pseudomonas stutzeri*	*Stenotrophomonas maltophilia*	NONE	1	0	-	09078
Klebsiella oxytoca	*Pseudomonas putida*	*Stenotrophomonas maltophilia*	NONE	1	0	-	09079
A. baumannii complex	*Staphylococcus aureus*		OXA	1	0	-	09008
Acinetobacter baumannii	*Stenotrophomonas maltophilia*		NONE	1	0	-	99081
Aeromonas caviae	*Escherichia coli*		NONE	1	0	-	08017
Aeromonas hydrophila	*Escherichia coli*		NONE	1	0	-	08040
Corynebacterium spp.	*Sphingomonas paucimobilis*		N/A	1	0	-	09048
Cupriavidus pauculus	*Stenotrophomonas maltophilia*		N/A	1	0	-	06147
Delftia acidovorans	*Stenotrophomonas maltophilia*		N/A	1	0	-	09065
E. cloacae complex	*Enterococcus faecium*		NONE	1	0	-	13040
E. cloacae complex	*Enterococcus* spp.		NONE	1	0	-	99105
E. cloacae complex	*Hafnia alvei*		NONE	1	0	-	17082
E. cloacae complex	*Klebsiella oxytoca*		NONE	1	1	*K. oxytoca*	08066
E. cloacae complex	*Morganella morganii*		NONE	1	0	-	09063
E. cloacae complex	*Pantoea* spp.		NONE	1	0	-	11055
Enterobacter aerogenes	*Escherichia coli*		CTX-M	1	1	CTX-M	11065
Escherichia coli	*Enterococcus faecalis*		CTX-M	1	0	-	17010
Escherichia coli	*Klebsiella pneumoniae*		NONE	1	1	*Klebsiella pneumoniae*	16029
Escherichia coli	*Klebsiella oxytoca*		N/A	2	2	*K. oxytoca* (2)	99222 99303
Escherichia coli	*Pseudomonas aeruginosa*		NONE	1	1	*Pseudomonas aeruginosa*	07009
Escherichia coli	*Enterococcus faecium*		NONE	1	0	-	13028
Klebsiella pneumoniae	*Pseudomonas aeruginosa*		N/A	2	1	*Pseudomonas aeruginosa*	12045 09002
Klebsiella oxytoca	*Serratia marcescens*		NONE	1	0	-	12072
Pseudomonas putida	*Staphylococcus hominis*		N/A	1	0	-	09088
Pseudomonas aeruginosa	*Sphingomonas paucimobilis*		IMP	1	0	-	99586
Serratia marcescens	*Achromobacter xylosoxidans*		N/A	1	0	-	09092
Serratia marcescens	*Staphylococcus hominis*		N/A	1	0	-	11027
Serratia odorifera	*Pantoea* spp.		N/A	1	0	-	12031
Stenotrophomonas maltophilia	*Enterococcus faecium*		N/A	1	0	-	11068
	TOTAL			***35***	***9***		

1 Defined as an analyte that was detected by the reference methods which should have been detected by **BC-GN** but was not.

Table 10: Summary of Genus/Group-level Test Performance versus Reference Method(s) – Stratified by Species

Acinetobacter Genus			
	Clinical	***Analytical*** (2)	
Organism	***% POS Agreement (95% CI)***		***No. of Strains***(3)
Combined *Acinetobacter*	98.2% 55/56 (90.5-100)	96.3% 104/108 (90.8-9.0)	36
Acinetobacter spp.	100% 1/1 (2.5-100)	NT(1)	-
baylyi	NT	100% 6/6 (54.1-100)	2
lwoffii	100% 5/5 (47.8-100)	100% 9/9 (66.4-100)	3
baumannii	100% 30/30 (88.4-100)	100% 24/24 (85.8-100)	6
baumannii complex	93.3% 14/15 (68.1-99.8)	NT	-
ursingii	100% 2/2 (15.8-100)	100% 3/3 (29.2-100)	
bereziniae	NT	100% 3/3 (29.2-100)	1
calcoaceticus	NT	100% 15/15 (78.2-100)	5
guillouiae	NT	100% 3/3 (29.2-100)	1
haemolyticus	NT	100% 15/15 (78.2-100)	5
johnsonii	NT	100% 9/9 (66.4-100)	3
junii	NT	100% 9/9 (66.4-100)	3
schindleri	NT	100% 3/3 (29.2-100)	1
radioresistens	100% 3/3 (29.2-100)	55.6% 5/9 (21.2-86.3)	3

Citrobacter Genus			
	Clinical	***Analytical*** (2)	
Organism	***% POS Agreement (95% CI)***		***No. of Strains***(3)
Combined *Citrobacter*	100% 49/49 (92.8-100)	100% 123/123 (97.1-100)	41
amalonaticus	100% 3/3 (29.2-100)	100% 15/15 (78.2-100)	5
*braakii**	100% 10/10 (69.2-100)	100% 3/3 (29.2-100)	1
freundii	100% 22/22 (84.5-100)	100% 15/15 (78.2-100)	5
koseri	100% 12/12 (73.5-100)	100% 15/15 (78.2-100)	5
youngae	2/2 100% (15.8-100)	100% 15/15 (78.2-100)	5
farmeri	NT	100% 6/6 (54.1-100)	2
gillenii	NT	100% 9/9 (66.4-100)	3
murliniae	NT	100% 9/9 (66.4-100)	3
rodentium	NT	100% 15/15 (78.2-100)	5
sedlakii	NT	100% 9/9 (66.4-100)	3
werkmanii	NT	100% 9/9 (66.4-100)	3

Enterobacter Genus			
	Clinical	***Analytical*** (2)	
Organism	***% POS Agreement (95% CI)***		***No. of Strains***(3)
Combined *Enterobacter*	97.5% 119/122 (93.0-99.5)	100% 87/87 (95.9-100)	29
cloacae	96.3% 26/27 (81.0-99.9)	100% 24/24 (85.8-100)	6
cloacae complex	100% 74/76 (90.8-99.7)	NT	-
aerogenes	100% 15/15 (78.2-100)	100% 15/15 (78.2-100)	5
asburaie	100% 4/4 (40.0-100)	100% 12/12 (73.5-100)	4
amnigenus	NT	100% 9/9 (66.4-100)	3
cancerogenus	NT	100% 15/15 (78.2-100)	5
hormaechei	NT	100% 9/9 (66.4-100)	3
ludwigii	NT	100% 3/3 (29.2-100)	1
nimipressuralis oryzae	NT	100% 3/3 (29.2-100)	1
Proteus Genus			
Combined *Proteus*	100% 58/58 (93.8-100)	100% 48/48 (92.6-100)	16
mirabilis	100% 58/58 (93.8-100)	100% 15/15 (78.2-100)	5
hauseri	NT	100% 6/6 (54.1-100)	2
myxofaciens	NT	100% 3/3 (29.2-100)	1
penneri	NT	100% 6/6 (54.1-100)	2
vulgaris	NT	100% 15/15 (78.2-100)	5

(1) "Not Tested" (2) Analytical Reactivity (Inclusivity) (Section D) (3) Each strain was tested in triplicate

Table 11: Detection of CTX-M, OXA, and KPC Resistance Markers Linked with Organisms, as determined by reference method (n=1266)

	CTX-M		***OXA***		***KPC***	
Organism	***Percent Agreement***		***Percent Agreement***		***Percent Agreement***	
	Positive (95% CI)	***Negative (95% CI)***	***Positive (95% CI)***	***Negative (95% CI)***	***Positive (95% CI)***	***Negative (95% CI)***
Acinetobacter spp.	--	100% 52/52 (93.2-100)	85.0% 17/20[c] (62.1-96.8)	96.9% 31/32 (83.8-99.9)	--	100% 52/52 (93.21-100)
Citrobacter spp.	--	98.0% 49/50 (89.4-100)	100% 1/1[d] (2.5-100)	100% 49/49 (92.8-100)	100% 1/1[g] (2.5-100)	100% 49/49 (92.8-100)
Enterobacter spp.	100% 9/9[a] (66.4-100)	100% 111/111 (96.7-100)	100% 2/2[e] (15.8-100)	100% 118/118 (96.9-100)	100% 1/1[h] (2.5-100)	100% 119/119 (97.0-100)
E. coli	98.8% 85/86 (93.7-100)	100% 424/424 (99.1-100)	100% 16/16 (79.4-100)	100% 494/494 (99.3-100)	100% 2/2 (15.8-100)	100% 508/508 (99.3-100)
Proteus spp.	--	100% 56/56 (93.6-100)	--	100% 56/56 (93.6-100)	--	100% 56/56 (93.6-100)
P. aeruginosa	--	100% 124/124 (97.1-100)	--	100% 124/124 (97.1-100)	100% 1/1 (2.5-100)	100% 123/123 (97.1-100)
K. oxytoca	100% 1/1 (2.5-100)	100% 59/59 (93.9-100)	100% 2/2 (15.8-100)	100% 58/58 (93.8-100)	100% 1/1 (2.5-100)	100% 59/59 (93.9-100)
K. pneumoniae	100% 56/56 (93.6-100)	100% 217/217 (98.3-100)	100% 22/22 (84.6-100)	100% 251/251 (98.5-100)	100% 45/45 (92.1-100)	100% 228/228 (98.4-100)
Polymicrobial samples	0% 0/1[b] (0-97.5)	100% 20/20 (83.2-100)	0% 1/1[f] (2.5-100)	100% 20/20 (83.2-100)	--	100% 21/21 (83.9-100)
TOTAL	98.7% 151/153 (95.4-99.8)	99.9% 1112/1113 (99.5-100)	95.3% 61/64 (86.9-99.0)	99.9% 1201/1202 (99.5-100)	100% 51/51 (93.1-100)	100% 1215/1215 (99.7-100)

a. Eight (8) *Enterobacter cloacae* complex and one (1) *Enterobacter cloacae*
b. One (1) *Escherichia coli* and *Enterobacter aerogenes*
c. Eleven (11) *Acinetobacter baumannii* and six (6) *Acinetobacter baumannii* complex and three (3) *Acinetobacter radioresistens*
d. One (1) *Citrobacter braakii*
e. Two (2) *Enterobacter cloacae*
f. One (1) *Escherichia coli* and *Acinetobacter baumannii* complex and *Enterococcus* spp.
g. One (1) *Citrobacter freundii*
h. One (1) *Enterobacter cloacae* complex

Table 12: Detection of VIM, NDM, and KPC Resistance Markers Linked with Organisms, as determined by reference method (n=1266)

	VIM		NDM		IMP	
Organism	Percent Agreement		Percent Agreement		Percent Agreement	
	Positive (95% CI)	Negative (95% CI)	Positive (95% CI)	Negative (95% CI)	Positive (95% CI)	Negative (95% CI)
Acinetobacter spp.	--	100% 52/52 (93.2-100)	100% 1/1 [d] (2.5-100)	100% 51/51 (93.0-100)	100% 5/5 [g] (47.8-100)	100% 47/47 (92.5-100)
Citrobacter spp.	100% 3/3 [a] (29.2-100)	100% 47/47 (92.5-100)	100% 2/2 [e] (15.8-100)	100% 48/48 (92.6-100)	100% 1/1 [h] (2.5-100)	100% 49/49 (92.8-100)
Enterobacter spp.	100% 10/10 [b] (69.2-100)	100% 110/110 (96.7-100)	100% 6/6 [f] (54.7-100)	100% 114/114 (96.8100)	100% 6/6 [i] (54.1-100)	100% 114/114 (96.8-100)
E. coli	100% 1/1 (2.5-100)	100% 509/509 (99.3-100)	100% 15/15 (78.2-100)	100% 495/495 (99.3-100)	100% 1/1 (2.5-100)	100% 509/509 (99.3-100)
Proteus spp.	--	100% 56/56 (93.6-100)	--	100% 56/56 (93.6100)	100% 2/2 [j] (15.8-100)	100% 54/54 (93.4-100)
P. aeruginosa	100% 2/2 (15.8-100)	100% 122/122 (97.0-100)	--	100% 124/124 (97.1-100)	100% 22/22 (84.6-100)	100% 102/102 (96.5-100)
K. oxytoca	--	100% 60/60 (94.0-100)	--	100% 60/60 (94.0-100)	--	100% 60/60 (94.0-100)
K. pneumoniae	100% 24/24 (85.8-100)	100% 249/249 (98.5-100)	100% 17/17 (80.5-100)	100% 256/256 (98.6-100)	100% 10/10 (69.2-100)	100% 263/263 (98.6-100)
Polymicrobial samples	100% 1/1 [c] (2.5-100)	100% 20/20 (83.2-100)	--	100% 21/21 (83.9-100)	0% 1/1 [k] (2.5-100)	100% 20/20 (83.2-100)
TOTAL	100% 41/41 (91.4-100)	100% 1225/1225 (99.7-100)	100% 41/41 (91.4-100)	100% 1225/1225 (99.7-100)	100% 48/48 (92.6-100)	100% 1218/1218 (99.7-100)

a. Three (3) *Citrobacter freundii*
b. Six (6) *Enterobacter cloacae* and four (4) *Enterobacter cloacae* complex
c. One (1) *Klebsiella pneumoniae* and *Enterobacter cloacae* complex
d. One (1) *Acinetobacter baumannii* complex
e. Two (2) *Citrobacter freundii*
f. Two (2) *Enterobacter cloacae* and four (4) *Enterobacter cloacae* complex
g. Three (3) *Acinetobacter baumannii* and one (1) *Acinetobacter baumannii* complex and one (1) *Acinetobacter lwoffii*
h. One (1) *Citrobacter braakii*
i. Two (2) *Enterobacter cloacae* and four (4) *Enterobacter cloacae* complex
j. Two (2) *Proteus mirabilis*
k. One (1) *Klebsiella pneumoniae* and *Enterobacter cloacae* complex

Table 13: Summary of Organisms Containing Single and Dual Resistance Markers

BC-GN Resistance Marker Target		***No. of Strains Tested***	***Species Containing the Resistance Marker***
Presence of a Single Resistance Marker	CTX-M	70	*Enterobacter cloacae* (3), *Escherichia coli* (56), *Klebsiella pneumoniae* (11)
	OXA	20	*Acinetobacter baumannii* (12), *Acinetobacter radioresistens* (2), *Escherichia coli* (3), *Klebsiella oxytoca* (1), *Klebsiella pneumoniae* (2),
	IMP	36	*Klebsiella pneumoniae* (4), *Proteus mirabilis* (2), *Pseudomonas aeruginosa* (22), *Citrobacter braakii* (1), *Acinetobacter lwoffi* (1), *Acinetobacter baumannii* (1), *Enterobacter cloacae* (5),
	VIM	33	*Citrobacter freundii* (3), *Enterobacter cloacae* (9), *Klebsiella pneumoniae* (19), *Pseudomonas aeruginosa* (2)
	KPC	43	*Enterobacter cloacae* (1), *Klebsiella oxytoca* (1), *Citrobacter freundii* (1), *Klebsiella pneumoniae* (39),*Pseudomonas aeruginosa* (1)
	NDM	9	*Escherichia coli* (2), *Citrobacter freundii* (2), *Enterobacter cloacae* (2), *Klebsiella pneumoniae* (3)
	TOTAL	**211 (70%)**	
Presence of Dual Resistance Markers	NDM/CTX-M	29	*Enterobacter cloacae* (3), *Escherichia coli* (12), *Klebsiella pneumoniae* (14)
	IMP/CTX-M	8	*Enterobacter cloacae* (1), *Escherichia coli* (1), *Klebsiella pneumoniae* (6)
	OXA/CTX-M	36	*Escherichia coli* (13), *Klebsiella pneumoniae* (20), *Klebsiella oxytoca* (1), *Enterobacter cloacae* (1), *Citrobacter braakii* (1),
	KPC/CTX-M	5	*Escherichia coli* (2), *Klebsiella pneumoniae* (3)
	VIM/CTX-M	4	*Escherichia coli* (1), *Enterobacter cloacae* (1), *Klebsiella pneumoniae* (2)
	VIM/KPC	3	*Klebsiella pneumoniae*
	OXA/IMP	3	*Acinetobacter baumannii*
	OXA/NDM	2	*Acinetobacter baumannii* (1), *Enterobacter cloacae* (1)
	TOTAL	**90 (30%)**	
GRAND TOTAL		***301***	

The results of **BC-GN** for KPC, OXA, NDM, VIM, and IMP (individually and combined) were compared to phenotypic antimicrobial susceptibility testing (AST) using Meropenem agar gradient diffusion for informational purposes only. The percent positive agreement (PPA) for this comparison was calculated as 100% x (TP/TP + FN). A true positive (TP) is positive by **BC-GN** for KPC, OXA, NDM, VIM, and/or IMP and resistant (R) or intermediate (I) to Meropenem. A false negative (FN) is negative by **BC-GN** for KPC, OXA, NDM, VIM, and/or IMP and resistant (R) or intermediate (I) to Meropenem. The negative percent agreement (NPA) for this comparison was calculated as 100% x (TN/TN + FP). A true negative (TN) is negative by **BC-GN** for KPC, OXA, NDM, VIM, and/or IMP and susceptible (S) to Meropenem. A false positive (FP) is positive by **BC-GN** for KPC, OXA, NDM, VIM, and/or IMP and susceptible (S) to Meropenem. Results are provided in **Table 14** for 993 of the 1266 isolates tested from the same linked dataset utilized for **Table 11** and **Table 12**. ***Carbapenem resistance in these organisms can be due to mechanisms other than acquisition of the KPC (bla_{IMP}), OXA (bla_{IMP}), NDM (bla_{IMP}), VIM (bla_{IMP}), or IMP (bla_{IMP}) gene(s).***

Table 14: **BC-GN** Performance for KPC, OXA, NDM, VIM, and IMP and all Resistance Markers Combined as Compared to Phenotypic Antimicrobial Susceptibility Testing using Meropenem Agar Gradient Diffusion

BC-GN Panel Analyte Detected	n	Positive and Negative Percent Agreement (PPA and NPA) Detection of Resistance Markers Versus Meropenem Agar Gradient Diffusion (Resistant (R) or Intermediate (I))											
		KPC		OXA		NDM		VIM		IMP		"Combined Result" Data Analysis Algorithm (KPC, OXA, NDM, VIM, and/or IMP detected)	
		PPA	NPA	PPA	NPA	PPA	NPA	PPA	NPA	PPA	NPA	PPA[a]	NPA[b]
Acinetobacter spp.	28	0% 0/8	100% 20/20	100% 8/8	90.0% 18/20	12.5% 1/8	100% 20/20	0% 0/8	100% 20/20	37.5% 3/8	100% 20/20	100% 8/8 (63.1-100)	90.0% 18/20 (68.3-98.8)
Citrobacter spp.	31	33.3% 1/3	100% 28/28	0% 0/3	100% 28/28	33.3% 1/3	100% 28/28	33.3% 1/3	92.9% 26/28	0% 0/3	100% 28/28	100% 3/3 (29.2-100)	92.9% 26/28 (76.5-99.1)
Enterobacter spp.	87	0% 0/5	100% 82/82	0% 0/5	100% 82/82	40.0% 2/5	100% 82/82	60.0% 3/5	96.3% 79/82	0% 0/5	96.3% 79/82	100% 5/5 (47.8-100)	97.7% 76/82 (84.8-97.3)
Proteus spp.	49	0% 0/1	100% 48/48	0% 0/1	100% 48/48	0% 0/1	100% 48/48	0% 0/1	100% 48/48	0% 0/1	100% 48/48	0% 0/1 (0-75.5)	100% 48/48 (92.6-100)
Escherichia coli	435	3.7% 1/27	99.8% 407/408	40.7% 11/27	99.5% 406/408	51.9% 14/27	100% 408/408	0% 0/27	100% 408/408	0% 0/27	100% 408/408	96.3% 26/27 (81.0-99.9)	99.3% 405/408 (97.9-99.9)
Klebsiella pneumoniae	225	47.4% 36/76	98.7% 147/149	5.3% 4/76	97.3% 145/149	19.7% 15/76	100% 149/149	28.3% 20/76	99.3% 148/149	4.0% 3/76	97.9% 146/149	98.7% 75/76 (92.9-99.9)	93.3% 139/149 (88.0-96.7)
Klebsiella oxytoca	50	100% 1/1	100% 49/49	0% 0/1	100% 49/49	0% 0/1	100% 49/49	0% 0/1	100% 49/49	0% 0/1	100% 49/49	100% 1/1 (2.5-100)	100% 49/49 (92.8-100)
Pseudomonas aeruginosa	88	3.1% 1/32	100% 56/56	0% 0/32	100% 56/56	0% 0/32	100% 56/56	6.3% 2/32	100% 56/56	3.1% 1/32	100% 56/56	12.5% 4/32 (3.5-29.0)	100% 56/56 (93.6-100)
Total	993	26.1% 40/153	99.6% 837/840	15.0% 23/153	99.1% 832/840	21.6% 33/153	100% 840/840	17.0% 26/153	99.3% 834/840	4.6% 7/153	99.3% 834/840	79.7% 122/153 (71.5-85.8)	97.1% 816/840 (95.8-98.2)

a) Carbapenem resistance in the organisms detected by **BC-GN** can be due to mechanisms other than acquisition of the KPC (bla_{KPC}), OXA (bla_{OXA}), NDM (bla_{NDM}), VIM (bla_{VIM}), or IMP (bla_{IMP}) gene(s).
b) Detection of KPC, OXA, NDM, VIM, or IMP resistance markers may not always infer resistance to carbapenems.

The results of **BC-GN** for CTX-M were compared to phenotypic antimicrobial susceptibility testing (AST) using Ceftazidime and Ceftriaxone agar gradient diffusion for informational purposes only. The percent positive agreement (PPA) for this comparison was calculated as 100% x (TP/TP + FN). A true positive (TP) is positive by **BC-GN** for CTX-M and resistant (R) or intermediate (I) to Ceftazadime only or resistant (R) or intermediate (I) to Ceftazadime and/or Ceftriaxone. A false negative (FN) is negative by **BC-GN** for CTX-M and resistant (R) or intermediate (I) to Ceftazadime only or resistant (R) or intermediate (I) to Ceftazadime and/or Ceftriaxone. The negative percent agreement (NPA) for this comparison was calculated as 100% x (TN/TN + FP). A true negative (TN) is negative by **BC-GN** for CTX-M and susceptible (S) to Ceftazadime only or susceptible (S) to Ceftazidime and Ceftriaxone. A false positive (FP) is positive by **BC-GN** for CTX-M and susceptible (S) to Ceftazadime only or susceptible (S) to Ceftazidime and Ceftriaxone. Results are provided in **Table 15** for 993 of the 1266 isolates tested from the same linked dataset utilized for **Table 11** and **Table 12**. ***ESBL resistance in these organisms can be due to mechanisms other than acquisition of the CTX-M (bla_{CTX-M}) gene.***

Table 15: **BC-GN** Performance for Detection of CTX-M as Compared to Phenotypic Antimicrobial Susceptibility Testing using Ceftazadime and Ceftriaxone Agar Gradient Diffusion

BC-GN Panel Analyte Detected	n[(a)]	Positive and Negative Percent Agreement (PPA and NPA) Detection of Resistance Markers Versus Ceftazadime Only or Ceftazidime and/or Ceftriaxone Agar Gradient Diffusion			
		Ceftazadime (R) or (I)		Ceftazadime (R) or (I) and/or Ceftriaxone (R) or (I)	
		PPA[(c)]	NPA[(d)]	PPA[(c)]	NPA[(d)]
Acinetobacter spp.	28	0% 0/10 (0-30.9)	100% 18/18 (81.5-100)	0% 0/25 (0-13.7)	100% 3/3 (29.2-100)
Citrobacter spp.	31	0% 0/10 (0-30.9)	100% 21/21 (83.9-100)	0% 0/11 (0-28.5)	100% 20/20 (83.2-100)
Enterobacter spp.	87	10.0% 3/30 (2.1-26.5)	100% 57/57 (93.7-100)	12.1% 4/33 (3.4-28.2)	98.2% 53/54 (90.1-100)
Proteus spp.[(b)]	49	0% 0/5 (0-52.2)	100% 44/44 (92.0-100)	0% 0/5 (0-52.2)	100% 44/44 (92.0-100)
Escherichia coli	435	81.2% 56/69 (69.9-90.0)	96.5% 353/366 (94.0-98.1)	82.1% 69/84 (72.3-89.7)	100% 351/351 (99.0-100)
Klebsiella pneumoniae	225	33.7% 34/101 (24.6-43.8)	98.4% 122/124 (94.3-99.8)	34.6% 36/104 (25.6-44.6)	100% 121/121 (97.0-100)
Klebsiella oxytoca	50	0% 0/7 (0-41.0)	100% 43/43 (91.8-100)	0% 0/7 (0-41.0)	100% 43/43 (91.8-100)
Pseudomonas aeruginosa[(b)]	88	0% 0/24 (0.0-14.3)	100% 64/64 (94.4-100)	0% 0/24 (0.0-14.3)	100% 64/64 (94.4-100)
Total	***993***	***36.3% 93/256 (30.4-42.6)***	***98.0% 722/737 (96.7-98.9)***	***37.2% 109/293 (31.7-43.0)***	***99.9% 699/700 (99.2-100)***

(a) Ceftazadime only and Ceftazadime and/or Ceftriaxone Agar Gradient Diffusion results are provided for 993 of the 1266 total isolates available.
(b) Only Ceftazidime results were calculated for *Proteus* spp. and *Pseudomonas aeruginosa*--Ceftriaxone results are not applicable per CLSI M100-S22.
(c) Ceftazadime only and/or Ceftriaxone resistance in the organisms detected by **BC-GN** can be due to mechanisms other than acquisition of the CTX-M (bla_{CTX-M}) gene.
(d) In vitro resistance to Ceftazadime or Ceftriaxone is not always demonstrated for specimens containing CTX-M.

B. Precision and Reproducibility

The ***Precision Study*** involved the testing of an 18-member panel, containing eight (8) unique specimens representing each target analyte detected by **BC-GN,** as well as two negative controls, one consisting of negative blood culture media only and the second containing an organism not detected by **BC-GN** (*Hafnia alvei)*. The composition of the panel is presented in **Table 16**, together with the final call rates, call accuracy and two-sided 95% confidence limits obtained for the study. The 18-member panel was tested in-house by Nanosphere twice daily by two operators on twelve (12) non-consecutive days for a total of forty-eight (48) replicates per specimen. Except for the negative controls, organisms were tested at Bottle Positivity (BP) and Bottle Positivity + 8 hours incubation (BP+8h).

A total of 864 initial tests were conducted. There were five (5) Pre-Analytical Errors; these tests were repeated and valid test results were obtained for a pre-AE rate of 0.6% (5/880). There were eleven (11) initial No Calls, which were repeated once. All but one of these repeats generated a valid result, yielding a final call rate for the study (number of valid tests/total tests conducted) of 863/864 = 99.9%.

There was one inaccurate call involving a *Klebsiella pneumoniae*/OXA/CTX-M specimen at the BP+8h time-point, whereby **BC-GN** unexpectedly detected “K. oxytoca”, in addition to the correct expected calls of “K. pneumoniae*”, “* OXA” and “CTX-M”, resulting in a call accuracy of 862/863 = 99.9% for the study.

Table 16: Precision Study Panel Composition and Test Results

Sample			***Expected Call(s)***	***Bottle Positivity***		***Bottle Positivity + 8 hours***	
Organism/Specimen	***Resistance Marker(s)***	***Source No.***		*Final Call Rate*	*Accuracy*	*Final Call Rate*	*Accuracy*
Negative Control – Blood Culture Media Only	N/A	N/A	Not Detected	100% (48/48) 92.6-100	100% (48/48) 92.6-100	-	-
Hafnia alvei	N/A	ATCC 13337	Not Detected	97.9% (47/48) 88.9-100	100% (47/47) 92.5-100	-	-
Acinetobacter baumanii	OXA	IHMA 128307	*Acinetobacter* spp. & OXA	100% (48/48) 92.6-100	100% (48/48) 92.6-100	100% (48/48) 92.6-100	100% (48/48) 92.6-100
Citrobacter freundii	VIM	IHMA 549813	*Citrobacter* spp. & VIM	100% (48/48) 92.6-100	100% (48/48) 92.6-100	100% (48/48) 92.6-100	100% (48/48) 92.6-100
Enterobacter cloacae	KPC	IHMA 550287	*Enterobacter* spp. & KPC	100% (48/48) 92.6-100	100% (48/48) 92.6-100	100% (48/48) 92.6-100	100% (48/48) 92.6-100
Escherichia coli	NDM	IHMA 449261	*Escherichia coli* & NDM	100% (48/48) 92.6-100	100% (48/48) 92.6-100	100% (48/48) 92.6-100	100% (48/48) 92.6-100
Klebsiella pneumoniae	OXA, CTX-M	JMI 18518	*Klebsiella pneumoniae* & OXA & CTX-M	100% (48/48) 92.6-100	100% (48/48) 92.6-100	100% (48/48) 92.6-100	97.9% (47/48) 88.9-100
Klebsiella oxytoca	CTX-M	IHMA 683079	*Klebsiella oxytoca* & CTX-M	100% (48/48) 92.6-100	100% (48/48) 92.6-100	100% (48/48) 92.6-100	100% (48/48) 92.6-100
Proteus mirabilis	N/A	ATCC 12453	*Proteus* spp.	100% (48/48) 92.6-100	100% (48/48) 92.6-100	100% (48/48) 92.6-100	100% (48/48) 92.6-100
Pseudomonas aeruginosa	IMP	IHMA 576602	*Pseudomonas aeruginosa* & IMP	100% (48/48) 92.6-100	100% (48/48) 92.6-100	100% (48/48) 92.6-100	100% (48/48) 92.6-100

The ***Reproducibility Study*** involved the testing of an 18-member panel, containing eight (8) unique specimens representing each target analyte detected by **BC-GN,** as well as two negative controls, one consisting of negative blood culture media only and the second containing an organism not detected by **BC-GN** (*Morganella morganii)*. The composition of the panel is presented in **Table 17**, together with the final call rates, call accuracy and two-sided 95% confidence limits obtained for the study. The 18-member panel was tested at three (3) external sites twice daily in triplicate on five (5) non-consecutive days for a total of 90 replicates per specimen. Except for the negative controls, organisms were tested at Bottle Positivity (BP) and Bottle Positivity + 8 hours incubation (BP+8h).

A total of 1620 initial tests were conducted. There were nine (9) Pre-Analytical Errors; these tests were repeated and valid test results were obtained for a pre-AE rate of 0.5% (9/1652). There were 23 initial No Calls, which were repeated once. All of these repeats generated a valid result, yielding a final call rate for the study (number of valid tests/total tests conducted) and accuracy of 100% (1620/1620).

Table 17: Reproducibility Test Results

Sample			***Expected Call(s)***	***Bottle Positivity***		***Bottle Positivity + 8 hours***	
Organism/Specimen	***Resistance Marker(s)***	***Source No.***		*Final Call Rate*	*Accuracy*	*Final Call Rate*	*Accuracy*
Negative Control – Blood Culture Media Only	N/A	N/A	Not Detected	100% 90/90 (96.0-100)	100% 90/90 (96.0-100)	-	-
Morganella morganii	N/A	ATCC 25830	Not Detected	100% 90/90 (96.0-100)	100% 90/90 (96.0-100)	-	-
Acinetobacter baumanii	OXA	IHMA 128307	Acinetobacter spp. & OXA	100% 90/90 (96.0-100)	100% 90/90 (96.0-100)	100% 90/90 (96.0-100)	100% 90/90 (96.0-100)
Citrobacter freundii	VIM	IHMA 549813	Citrobacter spp. & VIM	100% 90/90 (96.0-100)	100% 90/90 (96.0-100)	100% 90/90 (96.0-100)	100% 90/90 (96.0-100)
Enterobacter cloacae	KPC	IHMA 550287	Enterobacter spp. & KPC	100% 90/90 (96.0-100)	100% 90/90 (96.0-100)	100% 90/90 (96.0-100)	100% 90/90 (96.0-100)
Escherichia coli	NDM	IHMA 449261	Escherichia coli & NDM	100% 90/90 (96.0-100)	100% 90/90 (96.0-100)	100% 90/90 (96.0-100)	100% 90/90 (96.0-100)
Klebsiella pneumoniae	OXA, CTX-M	JMI 18518	Klebsiella pneumoniae & OXA & CTX-M	100% 90/90 (96.0-100)	100% 90/90 (96.0-100)	100% 90/90 (96.0-100)	100% 90/90 (96.0-100)
Klebsiella oxytoca	CTX-M	IHMA 683079	Klebsiella oxytoca & CTX-M	100% 90/90 (96.0-100)	100% 90/90 (96.0-100)	100% 90/90 (96.0-100)	100% 90/90 (96.0-100)
Proteus mirabilis	N/A	ATCC 12453	Proteus spp.	100% 90/90 (96.0-100)	100% 90/90 (96.0-100)	100% 90/90 (96.0-100)	100% 90/90 (96.0-100)
Pseudomonas aeruginosa	IMP	IHMA 576602	Pseudomonas aeruginosa & IMP	100% 90/90 (96.0-100)	100% 90/90 (96.0-100)	100% 90/90 (96.0-100)	100% 90/90 (96.0-100)

C. Analytical Sensitivity (Limit of Detection)

Analytical sensitivity or limit of detection (LOD) for **BC-GN** was determined for each **BC-GN** genus level target with tests of two representative species:

Acinetobacter: *Acinetobacter baumanii* (OXA), *Acinetobacter calcoaceticus*
Citrobacter: *Citrobacter freundii* (VIM), *Citrobacter koseri*
Enterobacter: *Enterobacter cloacae* (KPC), *Enterobacter aerogenes*
Proteus: *Proteus mirabilis*, *Proteus vulgaris*

For each **BC-GN** species target, one representative strain was tested, including: *Escherichia coli* (NDM), *Klebsiella pneumoniae* (OXA, CTX-M), *Klebsiella oxytoca* (CTX-M), and *Pseudomonas aeruginosa* (IMP).

The LOD was assessed and confirmed by using bacterial strains with established titers. By definition, the LOD is the lowest target concentration that both bacterial and resistance marker targets can be detected approximately 95% of the time. For each strain, the dilution series began with the sample obtained at "bottle positivity". The dilution series were prepared by using a diluent matrix that comprised blood culture broth containing charcoal, human blood, and a common commensal skin bacteria (*Staphylococcus epidermidis*) at a minimum concentration of ~10^7 CFU/mL. Each dilution was tested in replicates of four. The putative LOD was the lowest concentration level where all the replicates for the analyte were 'Detected'. Once the putative LOD was established, 20 replicate samples were tested to confirm the LOD.

The LOD's were determined to be:

Target	***LOD (CFU/mL)***
Acinetobacter spp.	4.0×10^5 to 4.6×10^6
Citrobacter spp.	6.9×10^6 to 1.3×10^7
Enterobacter spp.	4.1×10^6 to 1.1×10^7
Proteus spp.	1.9×10^5 to 7.7×10^5
Klebsiella pneumoniae	1.2×10^7
Klebsiella oxytoca	2.0×10^7
Escherichia coli	3.7×10^6
Pseudomonas aeruginosa	2.3×10^7

D. Analytical Reactivity (Inclusivity)

Analytical reactivity was determined for **BC-GN** by testing a panel of 195 strains of 44 different bacterial species covering the genetic diversity of each **BC-GN** target and antibiotic resistance markers, as summarized below for the species targets and in **Table 18** for the genus level targets:

- *Klebsiella oxytoca* (10)
- *Klebsiella pneumoniae* (25)
- *Pseudomonas aeruginosa* (13)
- *E. coli* (17) and *Shigella* spp. (8)

Table 18: Organisms Tested for Inclusivity amongst the **BC-GN** Test Genus Level Bacterial Targets

BC-GN Target	***Total No. of Strains Tested***	***Species Tested***	
		Name (No. of Strains)	*Total*
Acinetobacter spp.*	36	*A. baylyi* (2), *A. baumannii* (8), *A. bereziniae* (1), *A. calcoaceticus* (5), *A. guillouiae* (1), *A. haemolyticus* (5), *A. johnsonii* (3), *A. junii* (3), *A. lwoffi* (3), *A. radioresistens* (3), *A. schindleri* (1), and *A. ursingii* (1)	12
Citrobacter spp.	41	*C. amalonaticus* (2), *C. braakii* (5), *C. farmeri* (2), *C. freundii* (5), *C. gillenii* (3), *C. koseri* (5), *C. murliniae* (3), *C. rodentium* (5), *C. sedlakii* (3), *C. werkmanii* (3), and *C. youngae* (5)	11
Enterobacter spp*.	29	*E. aerogenes* (5), *E. amnigenus* (2), *E. asburiae* (4), *E. cancerogenus* (5), *E. cloacae* (8), *E. hormaechei* (3), *E. ludwigii* (1), and *E. nimipressuralis/E. orzae* (1)	8
Proteus spp.	16	*P. hauseri* (2), *P. mirabilis* (6), *P. myxofaciens* (1), *P. penneri* (2), and *P. vulgaris* (5)	5

* **BC-GN** does not detect *Acinetobacter tartarogenes*, *Enterobacter gergoviae, Enterobacter kobei*, and *Enterobacter pyrinus*.

Of these 195 strains, 79 contained one or more resistance markers (RMs) associated with 11 different bacterial species including a total of 38 strains containing CTX-M, 17 containing OXA, 12 containing IMP, 10 containing VIM, 10 containing KPC, and 9 containing NDM (see **Table 19**). Separately, *in silico* analysis was performed by aligning the assay probes for each of the strains/organisms against available GenBank sequence entries to ensure that **BC-GN** is able to detect these strains.

Table 19: Summary of RMs Detected by Wet testing or Predicted to be Detected based on *in silico* Analysis

Marker	***Traditional Subgroups***	***Detected by Wet Testing***		***Predicted to Be Detected based on In Silico Analysis[2]***	
		No. of Samples	*Type Tested*	*Types With Identical Probe Binding Sites to Wet Tested Types(1)*	*Types with in Silico Data Only*
CTX-M	CTX-M-1	146	*1, 3, 12, 15, 22, 28, 30, 55, 79*	*11, 23, 29, 32, 33, 36, 37, 42, 52, 54, 57, 58, 60, 61, 62, 66, 69, 71, 72, 80, 82, 88, 96, 101, 107, 109, 114, 116, 117, 133*	*10, 34, 53, 68, 108, 123, 132*
	CTX-M-2	6	*2, 31*	*4, 5, 6, 7, 20, 43, 44, 56, 59, 77, 92, 95, 97, 124, 131*	*74, 75, 76*
	CTX-M-8	3	*8*	-	*40, 63*
	CTX-M-9	34	*9, 14, 24, 27, 45*	*102, 104, 105, 106, 110, 111, 112, 113, 121, 122, 126, 13, 134, 16, 17, 18, 19, 21, 38, 46, 47, 48, 49, 50, 51, 64, 65, 67, 81, 83, 84, 85, 86, 87, 90, 93, 98, 99*	-
	CTX-M-25	1	*39*	*25, 26, 41, 89, 91, 94, 100*	*78*
IMP		59	*1, 4, 7, 8, 13, 15, 16, 18, 26, 27*	*2, 5, 6, 10, 11, 19, 20, 21, 24, 25, 28, 29, 30, 33, 37, 38, 40, 41, 42*	*3, 9, 12, 14, 22, 32, 34, 35*
KPC		61	*2, 3, 4, 5, 11*	*1, 6, 7, 8, 9, 10, 12, 13, 14*	-
NDM		50	*1, 4, 6*	*2, 3, 5, 7*	-
OXA	23	18	*23*	*27, 49, 73, 133, 146, 165, 166, 167, 168, 169, 170, 171, 225, 239*	-
	40	5	*24, 40*	*25, 26, 33, 72, 139, 160, 207*	*143, 182, 231*
	48	48	*48, 162*	*163, 181, 199, 204, 232*	-
	58	7	*58*	*96, 97, 164*	-
VIM		51	*1, 2, 4, 5, 7, 26, 27, 28, 33*	*3, 6, 8, 9, 10, 11, 12, 14, 15, 16, 17, 18, 19, 20, 23, 24, 25, 29, 30, 31, 32, 34, 35, 36, 37*	*13*

(1) The **BC-GN** probe binding sites in these types are identical to those in the wet tested types; therefore, **BC-GN** performance for these types is expected to be the same as the wet tested types.

(2) ***These specific resistance marker types and subtypes were evaluated by in silico analysis only.***

The resistance makers in **Table 19** above were associated with the bacterial species identified in **Table 20** and **Table 21,** which provide a listing of the resistance markers linked to organisms that were wet tested during the Clinical Study and Analytical Studies, respectively. Please refer to **Table 11** and **Table 12** for related performance data. **Table 22** provides a summary of the organisms tested in the inclusivity study that contained single and dual resistance markers.

Table 20: Summary of Resistance Marker Types Linked to Organisms(1) Wet Tested – Clinical Study

BC-GN-Detected Linked Organism	***CTX-M***		***OXA***		***KPC***		***VIM***		***NDM***		***IMP***	
	n	*Types (#)(1)*	*n*	*Types (#)*	*n*	*Types (#)*	*n*	*Types (#)*	*n*	*Types (#)*	*n*	*Types (#)*
A. baumanii (2)	-	-	15	*23*	-	-	-	-	1	*1*	4	*1(2), 4*
A. lwoffii	-	-	-	-	-	-	-	-	-	-	1	-
A. radioresistens	-	-	2	-	-	-	-	-	-	-	-	-
C. braakii	-	-	1	*48*	-	-	-	-	-	-	1	-
C. freundii	-	-	-	-	1	*3*	3	*1(2), 2*	2	*1(2)*	-	-
E. cloacae(2)	9	*3, 14, 15(3)*	2	*48*	1	-	10	*1(7), 4*	6	*1(5)*	6	-
E. coli	85	*1, 15(22), 27, 55*	16	*48(16)*	2	*2(2)*	1	*1*	15	*1(11), 6*	1	-
K. oxytoca	1	*15*	2	*48(2)*	1	*2*	-	-	-	-	-	-
K. pneumoniae	56	*15(38), 27(3)*	22	*48(21)*	45	*2(22), 3(4), 11(2)*	24	*1(16), 26(3), 33*	17	*1(16)*	10	*26(3)*
P. mirabilis	-	-	-	-	-	-	-	-	-	-	2	*27(2)*
P. aeruginosa	-	-	-	-	1	*5*	2	*1*	-	-	22	*7(2), 13*
Polymicrobial	-	-	1(3)	-	-	-	1(4)	-	-	-	1(4)	-

(1) Only accounts for specimens for which specific resistance marker type identification information was available
(2) Includes organisms identified as *A. baumanii* complex or *E. cloacae* complex, respectively
(3) *Escherichia coli* and *Acinetobacter baumannii* complex and *Enterococcus spp.*
(4) *Klebsiella pneumoniae* and *Enterobacter cloacae* complex

Table 21: Summary of Resistance Marker Types Linked to Organisms(1) Wet Tested – Analytical Studies

Linked Organism	***CTX-M***		***OXA***		***KPC***		***VIM***		***NDM***		***IMP***	
	n	Types (#)(2)	n	Types (#)	n	Types (#)	n	Types (#)	n	Types (#)	n	Types (#)
A. baumanii(3)	-	-	8	*23(4), 24/40, 58(3)*	-	-	1	*2*	-	-	-	-
A. lwoffii	-	-	1	*58*	-	-	-	-	-	-	-	-
A. radioresistens	-	-	3	*23(3)*	-	-	-	-	-	-	-	-
C. freundii	1	*9*	-	-	2	*2, 3*	1	-	-	-	-	-
E. cloacae(3)	5	*2, 9, 12, 15, 30*	-	-	1	-	1	*5*	1	*1*	-	-
E. hormaechei	-	-	-	-	1	-	-	-	-	-	-	-
E. coli	17	*1(2), 2, 3, 8(2), 14, 15(5), 24, 27, 28, 55*	2	*48(2)*	-	-	-	-	5	*1(2), 4(2), 6*	1	*1*
K. oxytoca	3	*14, 31, 34*	-	-	-	-	-	-	-	-	-	-
K. pneumoniae	13	*1(4), 8, 12, 14, 15(3), 22, 39, 79*	3	*48(2), 162*	6	*2(2), 3, 4, 11(2)*	3	*1, 26, 27*	3	*1(2)*	4	*8, 26(2)*
P. mirabilis	-	-	-	-	-	-	-	-	-	-	1	*27*
P. aeruginosa	-	-	-	-	-	-	4	*1, 2, 7, 28*	-	-	6	*1, 7, 15*

(1) Several organisms were shown to contain two or more resistance markers; these are accounted for separately in this table
(2) Only accounts for specimens for which specific resistance marker type identification information was available
(3) Includes organisms identified as *A. baumanii* complex or *E. cloacae* complex, respectively

Table 22: Organisms Tested for Inclusivity amongst the **BC-GN** Test Resistance Marker Targets

BC-GN Resistance Marker Target		***Total No. Strains Tested***	***Species Tested Containing the Resistance Marker***
Presence of a Single Resistance Marker	CTX-M	22	*Citrobacter freundii* (1), *Enterobacter cloacae* (4), *Escherichia coli* (9), *Klebsiella oxytoca* (3), *Klebsiella pneumoniae* (5)
	OXA	11	*Acinetobacter baumannii* (7), *Acinetobacter lwoffi* (1), *Acinetobacter radioresistens* (3)
	IMP	10	*Klebsiella pneumoniae* (3), *Proteus mirabilis* (1), *Pseudomonas aeruginosa* (6)
	VIM	10	*Acinetobacter baumannii* (1), *Citrobacter freundii* (1), *Enterobacter cloacae* (1), *Klebsiella pneumoniae* (3), *Pseudomonas aeruginosa* (4)
	KPC	9	*Enterobacter cloacae* (1), *Enterobacter hormaechei* (1), *Citrobacter freundii* (2), *Klebsiella pneumoniae* (5)
	NDM	1	*Escherichia coli*
Presence of Dual Resistance Markers	NDM/CTX-M	8	*Enterobacter cloacae* (1), *Escherichia coli* (4), *Klebsiella pneumoniae* (3)
	IMP/CTX-M	2	*Escherichia coli* (1), *Klebsiella pneumoniae* (1)
	OXA/CTX-M	5	*Escherichia coli* (2), *Klebsiella pneumoniae* (3)
	KPC/CTX-M	1	*Klebsiella pneumoniae* (1)

All strains were grown to bottle positivity in blood culture bottles in automated blood culture instruments. Samples were tested for purity and organism concentrations were determined by colony count (CFU/mL). Samples were tested in triplicate with **BC-GN**.

The overall accuracy of **BC-GN** for the detection of all bacterial and resistance marker targets tested was 98.3% (616/627). False negative test results were observed, specific to the detection of the OXA resistance marker present in two strains of *Acinetobacter radioresistens.* Of the 13 strains tested containing OXA, tested in triplicate, OXA was detected at a rate of 100% for all but two strains of *A. radioresistens* [ATCC 43999 (initial 2/3 replicates; repeat testing 5/9 replicates for a total of 7/12 replicates), ATCC 49000 (initial 0/3 replicates; repeat testing 8/9 replicates for a total of 8/12 replicates)]; however, in both cases, the bacterial target was correctly identified by **BC-GN** as "Acinetobacter".

Additionally, amongst the two strains each of *Citrobacter amalonaticus* and *Citrobacter farmeri* tested, only one strain of *Citrobacter amalonaticus* was not detected at a rate of 100% upon replicate testing (ATCC 25405, [initial 3/3 replicates; repeat testing 4/6 replicates for a total of 7/9 replicates]). However, *in silico* analysis suggested that both of these species could potentially yield false negative results with **BC-GN** based upon probe sequence homology mismatches (see "Limitations").

Eight *Shigella* strains representing four species were tested including *S. boydii* (2), *S. dysenteriae* (2), *S. flexneri* (2), and *S. sonnei* (2). All were detected by **BC-GN** as "E. coli Detected".

E. Analytical Specificity (Exclusivity)

Analytical specificity was assessed using organisms phylogenetically related to panel organisms detected by **BC-GN**, organisms with unknown genomes, common blood-borne pathogens, as well as organisms potentially present as contaminants in blood culture specimens The exclusivity samples were divided into two distinct panels of organisms.

The first panel consisted of 172 "non-**BC-GN** panel" organisms, which were not expected to be detected by **BC-GN**, including:

- Eighty-eight (88) gram-negative bacteria including *Acinetobacter baumanii* containing OXA-51 (OXA-51 is not detected by **BC-GN**),
- Seventy-one (71) gram-positive bacteria,
- Six (6) gram-negative cocci bacteria, and
- Seven (7) yeast strains

Of the 172 strains tested, 159 demonstrated no cross-reactivity with **BC-GN** while thirteen organisms were determined to cross-react with **BC-GN** panel analytes, as listed in **Table 23**. Specimens containing *Shigella* spp. or *E. coli* will be reported as "E. coli detected". See "Limitations" section for additional information. See **Tables 24** to **27** for a more detailed listing of the organisms tested.

Table 23: Cross-Reactivity Test Results

BC-GN Target for Which Cross Reactivity Observed	*Cross Reactive Organism/Resistance Markers*
Citrobacter spp.	*Buttiauxella gaviniae*
	Enteric group 137
Enterobacter spp.	*Klebsiella variicola*
	Leclercia adecarboxylata
Escherichia coli	*Escherichia albertii*
	S. dysenteriae
	S. flexneri
	S. boydii
	S. sonnei
Klebsiella oxytoca	*Kluyvera ascorbata*
	Raoultella ornithinolytica
	Raoultella planticola
	Cedecea davisae
CTX-M	*Kluyvera georgiana**
	Leminorella grimontii
	Enterococcus raftinosus
	Candida parapsilosis
	blaKLUA
	blaKLUG
	blaKLUY

*Organism confirmed by bi-directional sequencing to contain CTX-M

The second panel tested for exclusivity consisted of the 195 "**BC-GN** panel" organisms representing 44 different bacterial species listed in **Section D** above, which in total comprised the analytical inclusivity study samples and no cross-reactivity was observed between the panel members.

Table 24: Gram Negative Organisms Tested

Genus	Species
Acinetobacter	*tartarogenes*
Aggregatibacter	*aphrophilus*
Bacteroides	*fragilis*
	ovatus
	uniformis
	thetaiotamicron
Brevundimonas	*diminuta*
	vesicularis
Burkholderia	*cepacia*
Buttiauxella	*gaviniae*
Capnocytophaga	*ochracea*
Cardiobacterium	*hominis*
Cedecea	*davisae*
	lapagei
	neteri
Comamonas	*testosteroni*
Cronobacter	*sakazakii*
	muytjensii
Delftia	*acidovorans*
Eikenella	*corrodens*
Edwardsiella	*tarda*
Enteric group 137	*Enteric group 137*
Enterobacter	*gergoviae*
	kobei
	pyrinus
Escherichia	*albertii*
	blattae
	fergusonii
	hermannii
	vulneris
Elizabethkingia	*meningoseptica*
Ewingella	*americana*
Fusobacterium	*necrophorum*
Fusobacterium	*nucleatum*
Hafnia	*alvei*
Haemophilus	*influenzae*
	parainfluenzae
Herbaspirillum	*huttiense*
Kingella	*kingae*
Klebsiella	*variicola*
Kluyvera	*ascorbata*
	cryocrescens
	georgiana
Leclercia	*adecarboxylata*
Leminorella	*grimontii*
	richardii
Morganella	*morganii*
Pantoea	*agglomerans*
Parabacteroides	*distasonis*
	merdae
Pasteurella	*aerogenes*
	multocida
Plesiomonas	*shigelloides*
Prevotella	*bivia*
	melaninogenica
	buccae
	denticola
Providencia	*alcalifaciens*
	rettgeri
	stuartii
Pseudomonas	*alcaligenes*
	chloraphis
	fragi
	fulva
	fluorescens
	luteola
	mendocina
	mucidolens
	nitroreducens
	pertucinogena
	pseudoalcaligenes
	putida
	stutzeri
	veronii
Raoultella	*ornithinolytica*
	planticola
Salmonella	*bongori*
	enterica subsp enterica serovar Bareilly
	enterica subsp enterica serovar Typhimurium
Serratia	*fonticola*
	liquefaciens complex
	marcescens
	odorifera
Stenotrophomonas	*maltophilia*

Table 25: Gram Positive Organisms Tested*

Genus	Species
Aerococcus	*viridans*
Arcanobacterium	*bernardiae*
	haemolyticum
Bacillus	*cereus*
	licheniformis
	sphaericus
	subtilis
	thuringiensis
Cellulosimicrobium	*cellulans*
Cellumonas	*turbata*
Clostridium	*bifermentans*
	clostridioforme
	perfringens
	septicum
	tertium
Corynebacterium	*bovis*
	diphtheriae
	flavescens
	genitalium
	glutamicum
	jeikeium
	renale
	striatum
	urealyticum
Enterococcus	*avium*
	casseliflavis
	durans
	faecalis
	faecium
	flavescens
	gallinarum
	hirae
	mundtii
	raffinosus
Erysipelothrix	*rhusiopathiae*
Finegoldia	*magna*
Kocuria	*kristinae*
Kytococcus	*sedentarius*
Lactobacillus	*acidophilus*
	crispatus
	rhamnosus
Leuconostoc	*carnosum*
	mesenteroids
Listeria	*monocytogenes*
Micrococcus	*luteus*
Parvimonas	*micra*
Pediococcus	*acidilactici*
	pentosaceus
Peptostreptococcus	*anaerobius*
Planococcus	*citreus*
	kocurii
Propionibacterium	*acnes*
Rothia	*dentocariosa*
Rothia (Stomatococcus)	*mucilaginosa*
Staphylococcus	*aureus*
	caprae
	epidermidis
	haemolyticus
	hominis
	intermedius
	lugdunensis
	schleiferi
Streptococcus	*agalactiae*
	anginosus
	constellatus
	equinus
	intermedius
	pneumoniae
	pyogenes

Table 26: Gram Negative Cocci Tested

Genus	Species
Moraxella	*catarrhalis*
Neisseria	*lactamica*
	mucosa
	sicca
	meningitidis
Veillonella	*parvula*

Table 27: Yeast Organisms Tested

Genus	Species
Candida	*albicans*
	glabrata
	krusei
	parapsilosis
	tropicalis
Cryptococcus	*neoformans**
Saccharomyces	*cerevisiae*

*Genomic DNA Tested

F. Interfering Substances

The potential inhibitory effects of substances that may be encountered in blood and associated with the blood culturing process were tested with **BC-GN** at biologically or experimentally relevant concentrations. The interference testing was conducted by adding potential interferents present in patient blood specimens directly into blood cultures containing the bacterial strains and testing the resulting samples with **BC-GN**.

Representative strains, most with resistance markers, of *A. baumannii* (OXA), *C. freundii* (VIM), *E. cloacae* (KPC), *E. coli* (NDM), *K. pneumoniae* (OXA, CTX-M), *K. oxytoca* (CTX-M), *P. mirabilis*, and *P. aeruginosa* (IMP) were challenged with hemoglobin (14 g/L), triglycerides (3000 mg/dL), conjugated and unconjugated bilirubin (20 mg/dL), gamma-globulin (6 g/dL), and Sodium Polyanetholesulfonate (SPS, 0.25% w/v) at concentrations approximately one log higher than reference levels (except SPS). A negative control consisting of blood culture media was also tested. None of the added interferents were found to have an impact on the performance of **BC-GN**.

G. Carryover/Cross-Contamination Study

A study was performed using twelve Verigene Processor *SP* instruments to assess the potential for carryover/cross-contamination with **BC-GN** by alternately running “high positive” samples followed by negative samples. Representative strains, several with resistance markers, of *A. baumannii* (OXA), *C. freundii* (VIM), *C. sedlakii, C. koseri, E. cloacae* (KPC), *E. aerogenes*, *E. coli* (NDM), *K. pneumoniae* (CTX-M, OXA), *K. oxytoca*, *P. mirabilis*, and *P. aeruginosa* (IMP) were used to prepare the high positive samples. All of the high positive samples yielded the expected “Detected” results for the intended bacteria and “Not Detected” results for the other analytes. The negative samples gave a ‘Not Detected” call for all analytes. The studies confirmed that there was no evidence of carryover/cross-contamination from the high positive samples, or any other internal or external sources.

H. Universal Blood Culture Bottle Validation

The performance of **BC-GN** was evaluated for thirteen (13) types of blood culture media using three different automated blood culture monitoring systems (see listing of culture bottles tested below). This evaluation demonstrated that **BC-GN** performed appropriately when target organisms grew to a sufficient concentration in the various bottle types and that the bacterial DNA is sufficiently stable over 36 hours once bottle positivity has been reached. Three organisms, however, did not grow in all of the bottles tested. Expectedly, *A. baumannii* and *P. aeruginosa* did not grow in any of the anaerobic bottles, as these two organisms are obligate aerobes. Since these observations are related to the ability of the organisms to grow in specific blood culture bottles, they do not reflect on the performance of **BC-GN**. Furthermore, *A. baumannii* repeatedly did not grow in one aerobic bottle (VersaTREK REDOX 1 EZ Draw /Aerobic); therefore, the performance of BCGN for this organism in this bottle is unknown.

BACTEC™	***BacT/ALERT®***	***VersaTREK®***
Plus/Aerobic/F	SA Standard Aerobic	REDOX 1 EZ Draw® Aerobic
Plus/Anaerobic/F	FA FAN Aerobic	REDOX 2 EZ Draw® Anaerobic
Standard/10 Aerobic/F	PF Pediatric FAN	
Peds Plus/F	SN Anaerobic	
Standard Anaerobic/F	FN FAN® Anaerobic	
Lytic/10 Anaerobic/F		

Representative **BC-GN** bacterial organisms (all eight bacteria and six resistance markers) were inoculated into each of the different bottle types which were spiked with anti-coagulated human whole blood. A total of 1062 **BC-GN** tests were performed in the study, with an overall call accuracy of 99.8%, due to the observation of two OXA false negative results with *A. baumannii* using the BACTEC Standard Aerobic/F bottles. These false negative results were not attributable to the BACTEC Standard Aerobic/F bottle in which the organism was grown, but due to a limitation of **BC-GN** whereby in rare instances the organism may be detected but not the resistance marker. Therefore, with the exceptions noted, the results demonstrated that these thirteen (13) blood culture bottles are appropriate for use with **BC-GN** and that specimens are stable in those bottles at refrigerated (2-8 °C), ambient (18-24 °C), and culture system (34-37 °C) storage conditions for up to 36 hours after reaching bottle positivity

I. Competitive Inhibition/ Mixed Cultures

A competitive inhibition study was conducted to evaluate the impact of mixed cultures on **BC-GN** performance. Combinations of eight organisms with resistance markers representing the **BC-GN** panel targets (for a total of 28 paired combinations) were co-inoculated into individual blood culture bottles at clinically-relevant starting concentrations, and incubated to positivity. **BC-GN** correctly detected the bacteria and resistance marker(s) for four of the eight (8) target organisms together with their associated resistance marker, irrespective of combination, present in co-inoculated blood culture bottles (*A. baumannii*/OXA, *C. freundii*/VIM, *K. pneumoniae*/OXA and CTX-M, and *P. mirabilis*), demonstrating that these organisms are not subject to competitive inhibition at concentrations expected in routine clinical practice. For the remaining four organisms, at least one of the expected bacterial or resistance marker targets was not detected. This was due most likely to the slower growth rates of these organisms relative to the other co-infected organisms, as faster growing organisms may reach a higher concentration at bottle positivity compared with a slower growing organism. This may lead to slower-growing organism concentrations that are below the limit of detection of the test.

A second competitive inhibition study was conducted to further evaluate representative combinations of the four (4) organisms for which at least one false negative result was observed during the first study. This involved retesting nine (9) specific organism combinations:

Primary Organism	***Secondary Organism***	***Combination No.***
E. coli (NDM)	*K. pneumoniae* (CTX-M/OXA)	1
K. oxytoca (CTX-M)	*A. baumanii* (OXA)	2
	E. coli (NDM)	3
	K. pneumoniae (CTX-M/OXA)	4
	P. mirabilis	5
P. aeruginosa (IMP)	*A. baumanii* (OXA)	6
	E. cloacae (KPC)	7
	E. coli (NDM)	8
	K. oxytoca (CTX-M)	9

This study was designed to confirm whether false negative results observed with these combinations were due to the slower growth rates of the undetected organisms relative to the detected organisms. Growth rate was eliminated as an experimental variable by testing mixed culture organisms at “bottle positivity” concentrations and above the LOD for each organism. Of the nine (9) combinations, eight (8) yielded expected calls. For one combination, *K. oxytoca*/CTX-M and *E. coli*/NDM, the *K. oxytoca* target was detected at a rate of 78% (7/9); and 100% detection was observed for the other three (3) targets in this sample (*E. coli*, NDM, and CTX-M). This demonstrated that except in one instance, growth rate, not competitive inhibition was a contributing factor to the initial observed false negative results.

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TEST KIT LABELING

The contents of a Test Kit may use EN 980 graphical symbols. The symbols are defined below.

Symbol	Definition
Rx Only	Caution: Federal Law restricts this device to sale by or on the order of a licensed practitioner
REF	Catalog number
	Use by YYYY-MM-DD
LOT	Batch code
SN	Serial number
IVD	In vitro diagnostic medical device
	Manufacturer
	Upper Limit – Temperature limitation
	Upper and Lower Limit – Temperature limitation
i	Consult instructions for use
i KEY-CODE	Key-code; Use this key-code to obtain instructions for use at www.e-labeling.eu
Xn	Harmful
F	Flammable

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